DEVELOPMENT AND CHARACTERISATION OF BUCCAL FILM OF HYDROCHLOROTHIAZIDE

Dissertation submitted in partial fulfillment of the
Requirement for the award of the degree of

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IN
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CHENNAI

DEPARTMENT OF PHARMACEUTICS
K.M.COLLEGE OF PHARMACY
UTHANGUDI, MADURAI - 625107
APRIL - 2014

CERTIFICATE

This is to certify that the dissertation entitled "**DEVELOPMENT AND CHARACTERIZATION OF BUCCAL FILM OF HYDROCHLOROTHIAZIDE"** submitted by **Mr. O.S.USMAN ALI**(**Reg.No.261210109**) in partial fulfilment for the award of Master of Pharmacy in Pharmaceutics under the TamilnaduDr.M.G.R Medical University, Chennai, done at **K.M.COLLEGE OF PHARMACY**, Madurai-625107, is a bonafide work carried out by him under my guidance and supervision during the academic year **APRIL 2014.**The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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We here by dedicate this little piece of work to almighty.

"The act of thanks giving does not exhibit ones sense of gratitude ,but the true tendency of leading a helping hand during emergency and the fact that every work has thousands of hands behind".

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ABBREVIATION

ABBREVIATION	EXPANDED FORM OF THE ABBREVATION
HCTZ	Hydrochlorothiazide
HPMC	Hydroxy propyl methyl cellulose
HEC	Hydroxyl ethyl cellulose
PMA	Percent moisture absorption
PML	Percent moisture loss
NaCMC	Sodium Carboxy methyl cellulose
FDA	Food drug administration
KDa	Kilodaltons
NMPT	Nuclear Medicine Perfusion Test
NLX	Naloxone
nm	Nanometer
h	hours

CONTENTS

1. INTRODUCTION

The buccal route has been used for many years to deliver drugs such as certain steroids that are subjected to first-pass metabolism. The buccal route has the advantage of allowing excellent accessibility, reasonable patient acceptance and compliance, avoids first-pass metabolism and involves a relatively robust mucosa[1].

Buccal delivery is defined as drug administration through the mucosal membranes lining the cheeks (buccal mucosa).

Based on our current understanding of biochemical and physiological aspects of absorption and metabolism of many biotechnologically - produced drugs, they cannot be delivered effectively through the conventional oral route. After oral administration many drugs are subjected to presystemic clearance extensive in liver, which often leads to a lack of significant correlation between membrane permeability, absorption, and bioavailability.

Difficulties associated with parenteral delivery and poor oral availability provided the impetus for exploring alternative routes for the delivery of such drugs. These include routes such as pulmonary, ocular, nasal, rectal, buccal, sublingual, vaginal, and transdermal. In absence of external stimuli to facilitate absorption, use of these alternative routes had limited success.

1.1. The oral cavity

The functions of the oral cavity (also referred to as the "buccal cavity")[2] includes the analysis of potential foodstuffs, mechanical processing, lubrication and digestion. The oral cavity consists of two regions, the outer oral vestibule which is bounded by the cheeks, lips, teeth and gingiva (gums) and the oral cavity proper which extends from the teeth and gums back to the fauces (which lead on to the pharynx) with the roof comprising the hard and soft palates. The tongue projects from the floor of the cavity. The buccal mucosa refers to the membrane lining the inside of the cheek, and the term "buccal drug delivery" refers to drug release which can occur when a dosage form is placed in the outer vestibule between the buccal mucosa and gingival.

The outer surface of the oral cavity is a mucous membrane consisting of an epithelium, basement membrane and lamina propria overlying a submucosa containing blood vessels and nerves. The mucosa can be divided into three types: the masticatory mucosa, found on the gingiva and hard palate; the lining mucosa found on the lips, cheek, floor of the mouth, undersurface of the tongue and the soft palate; and the specialised mucosa found on the upper surface of the tongue and parts of the lips. All consist of a squamous stratified epithelium, many cell layers thick (40-50 for the buccal mucosa) overlying a connective tissue layer, the lamina propria. In the case of the masticatory mucosa the outer layers are keratinised and may be said to be similar (but not identical) to skin. The total surface area of the oral mucosa is about 100 cm and the buccal mucosa makes up of about a third of this.

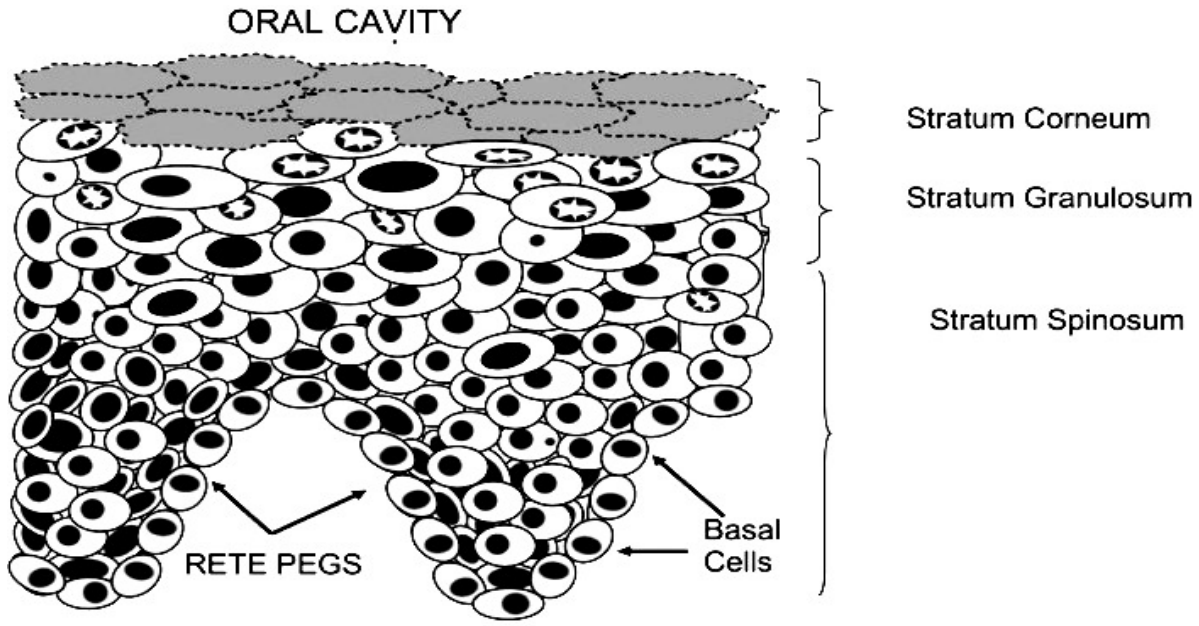


FIG 1. Cross sectional area of the buccal mucosa

The biochemistry of the oral mucosa, all the layers of the oral mucosa contain a large amount of protein in the form of tonafilaments, consisting of at least seven proteins called "keratins" with molecular sizes of 40 70 KDa. The difference between keratinised and non-keratinised epithelia is merely the difference in the molecular size of these keratins. Cells of nonkeratinised epithelia contain lower-molecular-weight proteins while those in keratinised epithelia contain mainly higher-molecular-weight keratins. The lipid content of the cells varies between tissues. The non-keratinised buccal and sublingual mucosa contain polar lipids while the keratinised gingival and palatal mucosa contain non-polar lipids. The intercellular material between the superficial epithelial layers is extruded by a unique organelle called a "membrane-coating granule". It has been shown in rat keratinised epithelium that the lamella contents of the membrane-coating granules mix with existing material and form broad sheets in the intercellular spaces. These sheets are orientated parallel to the cell membrane and therefore may act as a

barrier to permeability. The surface of the oral cavity is constantly bathed with a stream of saliva (approximately 1 liter per day) produced by the salivary glands. The major salivary glands, producing up to 90% of the saliva, are the pairs of parotid, sub maxillary (sub mandibular) and sublingual glands. The parotid glands are situated some way from, but drain into, the oral cavity via long ducts that open onto the inner surface of the cheek. The sub maxillary glands lie below the lower jaw and release saliva through ducts on each side of the floor of the mouth. The sublingual glands are located below the tongue with several ducts emptying onto the floor of the mouth.

The importance of saliva is illustrated in a condition called "xerostomia" (dry mouth) where patients complain of a variety of symptoms including sore mouth, oral infections, difficulty in talking, adhesion of the tongue to the side or roof of the mouth, and dental caries.

Chemically, saliva consists of 99.5% water with 0.5% solutes. The solutes include ions (sodium, potassium, calcium, magnesium, phosphate, bicarbonate and chloride), dissolved gases, urea, uric acid, serum albumin, globulin, mucin, and enzymes (lysozyme and amylase (ptyalin). The nature of the secretions varies from gland to gland; the parotid glands produce predominantly an amylase-containing watery secretion while the buccal and sublingual glands produce mainly a viscous saliva containing mucin with little enzymic activity. The submaxillary glands have an intermediate secretion containing both amylase and mucin. When food is ingested, secretion increases so that the saliva can lubricate, dissolve and bring about the chemical breakdown of food.

Saliva can be produced at a rate of up to 7 ml min- 1, 50% coming from the parotid gland. Thus the nature of the salivary secretion may alter from viscous to watery (and the enzyme content is also variable). The salivary pH will also vary from 6.2 to 7.4 (from low to high flow rates) although bacteria around the teeth may produce a lower localised pH.

The glycoproteins in saliva can be divided into two groups: those of mucous cell origin which have a high molecular weight and are heavily glycosylated and those of serous cell origin which have a lower molecular weight and contain less than 50% carbohydrate. The salivary mucin glycoprotein MG1 consists of several disulphide-linked subunits containing a protein core with 4-16 oligosaccharide side-chain units. Its molecular size is over 1000 kDa, and it contains approximately 15% protein, 78% carbohydrate with about 5-10% covalently bound fatty acids. A smaller mucin glycoprotein (MG2) has been identified from submaxillary and sublingual saliva. This contains 30% protein and 68% carbohydrate and has a molecular weight of 200-250 kDa. It consists of a single peptide chain with 2-7 oligosaccharide side-chain units. Another important glycoprotein found in human parotid saliva is proline-rich glycoprotein (PRG). This contains 60% protein and 30% carbohydrate and is 38.9 kDa in size.

Components of saliva are adsorbed onto the surface of the oral mucosa to form a salivary pellicle 0.1-0.7 mm thick. This pellicle coats all surfaces in the mouth and is a multilayered structure. Initially, salivary macromolecules are selectively adsorbed onto the mucosal surface, then these molecules complex with other molecules in the ambient saliva. It has been proposed that these salivary components may be covalently cross linked to the epithelial cell surface and to each other by the actions of transglutaminases. MG1 functions at the hard and soft tissue

interfaces to provide a permeability barrier for protection against environmental insult and desiccation. The nature of the salivary pellicle (i.e. its effectiveness in increasing the wet ability of a surface) has been seen to change throughout the day. The oral cavity contains large numbers of microorganisms and the salivary pellicle has been shown to be a determinant in bacterial adhesion.

Buccal Mucosa: Environment[3]

The oral cavity is marked by the presence of saliva produced by the salivary glands and mucus which is secreted by the major and minor salivary glands as part of saliva.

Role of Saliva

- Protective fluid for all tissues of the oral cavity.
- Continuous mineralization / demineralization of the tooth enamel .
- To hydrate oral mucosal dosage forms.

Role of Mucus

- Made up of proteins and carbohydrates.
- Cell-cell adhesion
- Lubrication
- Bioadhesion of mucoadhesive drug delivery systems

Permeability of Drugs Through Buccal Mucosa[4]

There are two possible routes of drug absorption through the squalors stratified epithelium of the oral mucosa:

i. Transcellular (intracellular, passing through the cell) and

ii. Paracellular (intercellular, passing around the cell).

Permeation across the buccal mucosa has been reported to be mainly by the paracellular route through the intercellular lipids produced by membrane-coating granules.

Although passive diffusion is the main mechanism of drug absorption, specialized transport mechanisms have been reported to exist in other oral mucosa (that of the tongue) for a few drugs and nutrients; glucose and cefadroxil were shown to be absorbed in this way.

The buccal mucosa is a potential site for the controlled delivery of hydrophilic macromolecular therapeutic agents (biopharmaceuticals) such as peptides, oligonucleotides and polysaccharides. However, these high molecular weight drugs usually have low permeability leading to a low bioavailability, and absorption enhancers may be required to overcome this.

The buccal mucosa also contains proteases that may degrade peptide-based drugs. In addition, the salivary enzymes may also reduce stability.

Disease states where the mucosa is damaged would also be expected to increase permeability. This would be particularly true in conditions that result in erosion of the mucosa such as lichen planus, pemphigus, viral infections and allergic reactions.

1.3. Drug delivery via the oral cavity[5]

The oral cavity can be used for local therapy (treatment of oral infections, dental caries, mouth ulcers, and stomatitis) and systemic therapy. The buccal route is of particular interest with regard to the systemic delivery of small molecules that are subjected to first-pass metabolism, or for the administration of proteins and peptides. The multilayered structure and mainly protective role of the mucosa within the oral cavity would imply that it would not be as good a site for drug absorption as other single cell layer mucosa, e.g. those found in the small and large intestines.

In keratinized oral mucosal tissue as with skin, the keratinized upper layer is the major barrier to drug absorption. The lamina propria is believed to offer little resistance to drug permeation. Regional variations in oral mucosal drug absorption are consistent with the differences in the thickness and composition of the mucosa within the oral cavity.

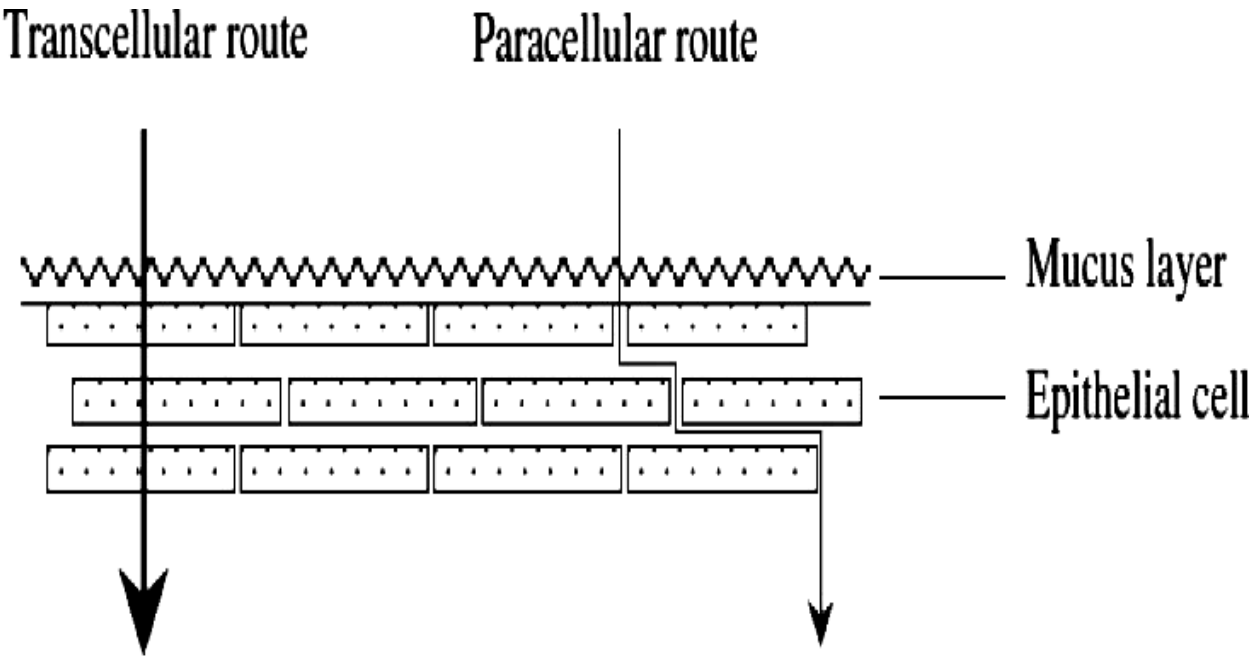


Fig 2. Schematic representation of penetration routes in buccal drug delivery.

The salivary pellicle may also act as a barrier for drug absorption. It is known that the salivary pellicle protects the mucosa from acids and enzymes, and there is evidence from animal studies that the absence of this layer allows the diffusion of various compounds through the mucosa, including acridine orange and benzpyrine.

The steady-state permeability coefficient for three peptides—thyrotrophin releasing hormone, DDAVP (1-deamino-8-D-arginine vasopressin) and insulin--have been calculated for the buccal route. The buccal mucosa was less permeable than nasal or intestinal mucosa, and only DDAVP gave calculated steady-state levels in excess of the therapeutic levels, due mainly to its comparatively long half-life. The use of penetration enhancers has been investigated as a suitable method for improving the penetration of non-peptide drugs through the buccal mucosa.

Laureth-9, sodium lauryl sulphate and steroidal detergents (e.g. sodium glycocholate) were found to be the most effective enhancers at pH 7.4 and their most effective formulations gave insulin levels one-quarter to one-third as effective as an intramuscular injection.

1.4. Advantages Buccal-adhesive dosage forms.

- Significant reduction in dose related side effects.
- It provides direct entry of drug into systemic circulation.
- Drug degradation in harsh gastrointestinal environment can be circumvented by administering the drug via buccal route.
- Drug absorption can be terminated in case of emergency.
- It offers passive system, which does not require activation.

- Rapid cellular recovery following local stress or damage.
- Ability to withstand environmental extremes like change in pH, temperature etc.
- Sustained drug delivery.
- The potential for delivery of peptide molecules unsuitable for the oral route.

1.5. Limitations

- Once placed at the absorption site, the dosage form should not be disturbed.
- Drugs which are unstable at buccal pH and which irritate the mucosa or have a bitter or unpleasant taste or an obnoxious odor cannot be administered by this route.
- Over hydration may lead to formation of slippery surface and structural integrity of formulation may get disrupted.

1.6. Mucosal-adhesive materials [6]

Mucosal-adhesive materials are generally hydrophilic macromolecules that contain numerous hydrogen-bond-forming groups. The presence of carboxyl groups and a molecular size greater than 100 kDa favor adhesion. In most cases these materials require moisture to become adhesive but may excessively hydrate to form slippery mucilage, and lose their adhesive properties. Several strategies (i.e. the inclusion of a hydrophobic component or a cross-linking agent) have been used to prevent excess hydration.

Some of the most extensively studied mucosal adhesives are the poly(acrylic acids), e.g. Carbopol 934 and polycarbophil. The high concentration of carboxyl groups in a dry tablet of poly(acrylic acid) would be predicted to generate a low surface pH on moistening, and pH values of between 2 and 3 have been detected in our laboratories. A low pH would be expected to

damage a contacting mucosal surface, and this has been reported in an *in vivo* study. Salts and bases have been included in poly(acrylic acid)-containing formulations to raise the pH, but the presence of predominantly ionised carboxyl groups would result in a loss of the adhesive properties. Thus the ultimate suitability of poly(acrylic acid) for use as a bioadhesive component in a pharmaceutical formulation may be questioned.

Other anionic mucosal-adhesive materials include sodium carboxymethylcellulose, sodium alginate, and maleic anhydride copolymers. Non-ionic polymers on the whole tend to be weaker adhesives, and these include hydroxypropylmethylcellulose, hydroxypropylcellulose, methylcellulose, poly(ethylene oxide), poly(vinyl alcohol), and starch. Chitosan and diethylaminoethyl-dextran are examples of cationic materials that have been proposed as mucosal-adhesive polymers.

1.6.2. Mechanism of mucosal adhesion[7]

Mucosal-adhesive materials are called "wet" adhesives in that they will adhere to most surfaces on moistening. The various theories of bioadhesion are the electronic theory, the adsorption theory, the wetting theory, the diffusion theory and the fracture theory. It has been envisaged that for dosage forms to adhere to mucous membranes, they must first interact with the overlying layer of mucus.

Mucoadhesion is proposed to occur in three stages. Initially, an intimate contact must form between the mucoadhesive and mucus (i.e. they must "wet" each other), then the mucus/mucoadhesive macromolecules interpenetrate, and finally the molecules interact with each other by secondary non-covalent bonds. It would be predicted that the mucus layer would

be the weakest component of the mucoadhesive joint. If interpenetration is an important stage in mucoadhesive bond formation, then including bioadhesive polymers into a mucus gel would be expected to increase its resistance to deformation, thus strengthening the adhesive joint.

With regard to the buccal cavity, dosage forms can be placed directly in position, and so force can be applied to ensure that the two surfaces come into intimate contact. Pressure will also be applied by the cheek and gums and it has been proposed that very little adhesive force is required to hold a dosage form in place.

Its main role in the adhesive process may therefore be to provide the moisture necessary for adhesion. Thus the surface of the oral cavity may be considered as a moist membrane to which these "wet" adhesives will attach, by predominantly Secondary interactions. Hydration is required to allow the molecules to attain a degree of flexibility to allow good contact with, and then interaction with, the epithelial cell surface. It is worth noting that wet cellophane, Visking tubing and other moist surfaces have been successfully used as a model for the oral mucosa in adhesion studies.

The existing interpenetration theory of mucoadhesion may not be appropriate for explaining the adhesion of dry dosage forms within the oral cavity. It may also be more appropriate to restrict the term "mucoadhesion" to describing the adhesion of hydrated dosage forms to those mucous membranes having a substantial mucus layer. The terms "bioadhesion" or "mucosal adhesion" may be more suitable to describe adhesion to the mucosa of the oral cavity.

1.7. Buccal-adhesive dosage forms[8]

The following requirements for buccal-adhesive dosage forms: (a) they should be flexible enough to follow the movement of the cheek; (b) they should be adhesive enough to be retained on the buccal mucosa but not so strong that the mucosa is damaged on removal; (c) they should be biocompatible and not cause irritation.

Buccal-adhesive dosage forms can deliver the drug either locally to treat conditions within the buccal cavity or systemically via the mucosa. It is often a requirement that buccal-adhesive dosage forms should remain adhesive and allow a controlled delivery of drug for prolonged periods. For systemic therapy, dosage forms can be designed to deliver drugs only to the associated section of adhering mucosa (in the buccal pouch this may be the buccal and gingival mucosa), or to release the drug into the saliva prior to absorption. When in place, the dosage form will need to withstand mechanical abrasion by the surrounding tissue, the effect of continued contact with saliva (although this should be less of a problem in the upper regions of the buccal pouch) and the presence of food and drink at mealtimes.

Therefore, for sustained drug delivery, buccal-adhesive formulations must contain elements that remain adhesive for a prolonged period, regulate the rate and direction of drug delivery. Another major problem with formulating dry dosage forms containing "wet" adhesives is that swelling occurs on hydration, and this may disrupt the integrity of these multilayered formulations.

Structure And Design Of Buccal Dosage Form [9]

Buccal Dosage form can be of

1**. Matrix type:** The buccal patch designed in a matrix configuration contains drug, adhesive, and additives mixed together

2. **Reserviour type:** The buccal patch designed in a reservoir system contains a cavity for the drug and additives separate from the adhesive. An impermeable backing is applied to control the direction of drug delivery; to reduce patch deformation and disintegration while in the mouth; and to prevent drug loss.

Additionally, the patch can be constructed to undergo minimal degradation in the mouth, or can be designed to dissolve almost immediately.

Transmucosal drug delivery systems can be bi-directional or unidirectional. Bi-directional (Figure 1) patches release drug in both the mucosa and the mouth while, Unidirectional (Figure 2) patches release the drug only into the mucosa.

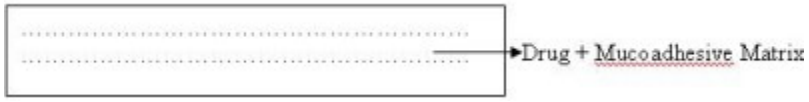


FIG 3 : Buccal Patch designed for Bidirectional drug release

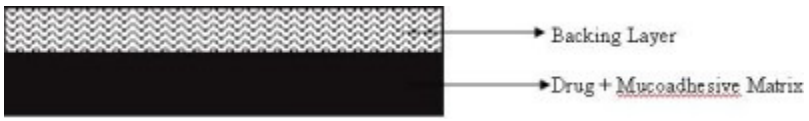


FIG 4: Buccal Patch designed for unidirectional drug release

1.7.1. Solid buccal adhesive dosage forms [10]

They are dry formulations which achieve bioadhesion via dehydration of the local mucosal surface.

(a). Buccal Tablets

Tablets have been the most commonly investigated dosage forms for buccal drug delivery. Several bioadhesive buccal tablet formulations have been developed by direct compression method in recent years either for local or systemic drug delivery. They are designed to release the drug either unidirectionally by targeting buccal mucosa or multi- directionally into the saliva. Alternatively, the dosage form can contain an impermeable backing layer to ensure that drug is delivered unidirectionally. A typical bioadhesive formulation of this type consists of a bioadhesive polymer (such as polyacrylic acids or a cellulose derivative), alone or in combination, incorporated into a matrix containing the active agent and excipients, and perhaps a second impermeable layer to allow unidirectional drug delivery.

(b). Bioadhesive Micro/nanoparticles

Bioadhesive micro/nanoparticles offer the same advantages as tablets but their physical properties enable them to make intimate contact with a lager mucosal surface area. These are typically delivered as an aqueous suspension or are incorporated into a paste or ointment or applied in the form of aerosols. Particulates have the advantage of being relatively small and more likely to be acceptable by the patients. Bioadhesive polymeric microparticles of carbopol, polycarbophil, chitosan or Gantrez are to adhere to porcine esophageal mucosa, with particles prepared from the polyacrylic acids exhibiting greater mucoadhesive strength during tensile testing studies. However in elution studies, particles of chitosan or Gantrez were found to persist

on mucosal tissue for longer periods of time. Moreover, the absolute bioavailability of microsphere formulations was higher than that of reference tablets in spite of a lower drug dose. Liposomes are one of the alternatives for drugs which are poorly soluble and hence are not efficiently delivered from a solid dosage form. For example, silamyrin liposomal buccal delivery showed steady state permeation through a chicken buccal pouch for 6 hrs and which was higher than free drug powder.

(c). Bioadhesive Wafers

The delivery system is a composite wafer with surface layers possessing adhesive properties, while the bulk layer consists of antimicrobial agents, biodegradable polymers and matrix polymers.

(d). Bioadhesive Lozenges

A slow release bioadhesive lozenge offers the potential for prolonged drug release with improved patient compliance. Bioadhesive lozenges may be used for the delivery of drugs that act within the mouth including antimicrobials, corticosteroids, local anaesthetics, antibiotics and antifungals. A Bioadhesive lozenge has been reported as a means to deliver antifungal agents to the oral cavity. The limitation of these bioadhesive lozenges is the short residence time at the site of absorption which depends to the size and type of formulation and since dissolve within 30min, the total amount of the drug that can be delivered is limited. The dissolution or disintegration of lozenges is usually controlled by the patient, i.e. how hard they suck the unit. Increased sucking and saliva production causes uncontrolled swallowing and loss of drug down the GI tract.

1.7.2.Semi-solid dosage forms

(a). Medicated chewing gums

Although medicated chewing gums pose difficulties in regulation of the administered dose, they still have some advantages as drug delivery devices, particularly in the treatment of diseases of the oral cavity and in nicotine replacement therapy. Some commercial products are available in the market. Caffeine chewing gum, Stay Alert, was developed recently for alleviation of sleepiness. It is absorbed at a significantly faster rate and its bioavailability was comparable to the capsule formulation. Nicotine chewing gums (e.g., Nicolette and Nicotine) have been marketed for smoking cessation.

(b). Adhesive Gels

Various adhesive gels may be used to deliver drugs *via* the buccal mucosa and allow sustained release. Gel forming bioadhesive polymers include cross- linked polyacrylic acid that has been used to adhere to the mucosal surfaces for extended periods of time and provide controlled release of drug at the site of absorption. The limitations for gel formulations are inability to deliver a measured dose of drug to the site and as a result have limited uses for drugs with narrow therapeutic window.

(c). Buccal patches/films

Patches are laminates consisting of an impermeable backing layer, a drug-containing reservoir layer from which the drug is released in a controlled manner, and a bioadhesive surface for mucosal attachment. Flexible films/patches have been prepared either by solvent casting or hot melt extrusion technique to deliver drugs directly to a mucosal membrane. Compared to creams and ointments they offer advantages in delivering a measured dose of drug to the site.

1.7.3. Liquid dosage forms

They are solutions or suspensions of drugs in suitable aqueous vehicles. Such types of dosage forms are usually employed to exert local action into the oral cavity and several antibacterial mouthwashes and mouth-freshener are commercially available for this purpose. The limitation associated with these liquid dosage forms are that they are not readily retained or targeted to buccal mucosa and can deliver relatively uncontrolled amounts of drug throughout oral cavity. From the wide range of polymer solutions, chitosan represents the greatest binding, followed by methylcellulose, gelatin, carbopol and polycarbophil. Viscous liquids may be used to coat buccal surface either as protectants or as drug delivery vehicles to the mucosal surface. Dry mouth is treated with artificial saliva solutions that are retained on mucosal surfaces to provide lubrication. These solutions contain sodium CMC as bioadhesive polymer.

Recent developments in buccal drug delivery systems

1. **Biobadhesive Spray**

Buccoadhesive sprays are gaining popularity over other dosage forms because of flexibility, comfort, high surface area and availability of drug in solution form. The fentanyl Oralet is the first FDA-approved formulation developed to take advantage of oral transmucosal absorption for the painless administration of an opioid in a formulation acceptable to children.

The FDA approved Subutex (buprenorphine) for initiating treatment of opioid dependence (addiction to opioid drugs, including heroin and opioid analgesics) and Suboxone (buprenorphine and naloxone) for continuing treatment of addicts. In 2005, Oral-lyn buccal spray was approved for commercial marketing and sales in Ecuador.

2. **Gel Forming Liquids:**

This type of a formulation is liquid upon instillation and undergoes a phase transition to form a viscoelastic gel in response to stimulus such as temperature, ionic strength or pH. Carbomers become more viscous upon increased pH. Poloxamers and smart hydrogel (Adnaced medical solution) gel at approximately body temperature.

Gellan gum and alginate both form gel in response to increased ionic strength (particularly with Ca^{+2} ions). Gel forming formulations are currently used for sustained ocular delivery. Recent work has examined the oesophageal retention of smart Hydrogel, a liquid that gels in response to both high force and temperature, with its gelling temperature at about 32°C.

For the delivery Recent developments in buccal drug delivery systems, such as lipophilic gel, buccal spray and phospholipid vesicles have been recently proposed to deliver peptides via the buccal route. In particular, some authors proposed the use of cubic and lamellar liquid crystalline phases of glyceryl monooleate as buccal drug carrier for peptide drugs. A novel liquid aerosol formulation (Oralin, Generex Biotec) has been developed recently. Phospholipid deformable vesicles, transfersomes, have been recently devised of insulin in the buccal cvaity.

1.8. Evaluation of Buccal Delivery Systems[11]

Buccal adhesive drug delivery devices are subjected to the routine evaluation tests such as weight variation, thickness variation, friability, hardness, content uniformity, *invitro* dissolution for tablets; tensile strength, film endurance, hygroscopicity etc. for films and patches; viscosity, effect of aging etc. for gels and ointments. They should also to be evaluated specifically for their bioadhesive strengths and permeabilities.

1.8.1. Moisture absorption studies for buccal patches

The moisture absorption studies for the buccal patches give an indication about the relative moisture absorption capacities of polymers and an idea whether the buccal patches maintain their integrity after absorption of moisture. Moisture absorption studies have been performed in 5 % w/v agar in distilled water, which while hot was transferred to plates and allowed to solidify. Then six buccal patches from each formulation were selected and weighed. Buccal patches were placed in desiccators overnight prior to the study to remove moisture if any and laminated on one side with water impermeable backing membrane. Placed on the surface of the agar plate and incubated at 37° C for 2 hrs in incubator.

Swelling and erosion studies for buccal tablets

Swelling and erosion studies for buccal tablets were determined gravimetrically in phosphate buffer, of pH 6.6. The tablets were attached to pre-weighed glass supports using a cyanoacrylate adhesive sealant. The supports with tablets were immersed into the phosphate buffer at 37 °C. At pre- determined time intervals, the devices were removed from the media, blotted with tissue paper to remove excess water, and weighed. After determination of the wet weight, the tablets were dried at 40°C until constant mass.

Study of the surface pH

The bioadhesive buccal tablets were covered with 1ml of distilled water and allowed to swell for 1-2h at room temperature. The surface pH of the tablets or patches was measured by bringing the pH meter electrode in contact with the surface of the patch or tablet and allowing it to equilibrate for one minute.

Bioadhesion measurement

Methods available for the measurement of bioadhesion are limited, and their selections depend on applicability, reproducibility, and providing useful information. It is unnecessary to compare the absolute values of different methods and is more meaningful to examine the relative bioadhesive performance using each technique. In addition, some factors, including saliva secretion, mastication, and mucus turnover that can markedly affect the adhesion strength and duration of *in vivo* adhesion are not present in *invitro* testing.

Determination of the residence time

***Ex vivo* residence time[12]**

Ex vivo residence time was determined using a modified USP disintegration apparatus by taking the disintegration medium composed of 800 ml phosphate buffer of pH 6.8 maintained at 37 °C. The porcine buccal tissue was tied to the surface of a glass slab, vertically attached to the apparatus. The time which was taken for complete erosion or detachment of the tablet from the mucosal surface was recorded and considered as *ex vivo* residence time.

***In vivo* residence time[13]**

The experiment was performed in eight healthy adult male volunteers, aged between 22 and 28 years. The volunteers were asked to record the residence time of the film on buccal mucosa in the oral cavity, which was taken as the time for the patch to dislodge completely from the buccal mucosa by continual sensation of the patch as well as the backing membrane. *In vivo* residence time was recorded in each case.

Permeation studies

Buccal absorption/permeation studies must be conducted to determine the feasibility of this route of administration for a drug candidate and to determine the type of enhancer and its concentration which were to control the rate of permeation of drugs during the pre-formulation studies. Similar to an *in vitro* permeation study in transdermal drug delivery, different types of diffusion cells with certain modifications are suitable to conduct permeation studies, except that the buccal mucosa dissected from model animals are used as diffusion barriers for buccal delivery. These studies involve methods that would examine *in vitro, ex vivo and/or in vivo* buccal permeation profile and kinetics of absorption of the drug.

Buccal absorption test

Measure the kinetics of the drug absorption by swirl a 25 ml sample of the test solution for 15 min by human volunteers followed by the expulsion of the solution. The amount of the drug remaining in the expelled volume is then determined to assess the amount of drug absorbed. The drawbacks of this method are inability to localize the drug solution within a specific site of the oral cavity, accidental swallowing of a portion of the sample solution and the salivary dilution of the drug.

Modified Beckett's test

The test has been modified by addition of phenol red as a marker for drug dilution by saliva secretion as well as for accidental swallowing of the drug solution. The 'Schumann and Turner Test' has also been modified by taking a small sample of the solution in the oral cavity every few minutes, without removal of the residual solution. In this way he was able to study kinetics of the absorption in a single test for 15–20 minutes. Advantages of this type of test over the original absorption test are; corrections for saliva secretion, accidental swallowing and changes in pH can be made and that a complete absorption curve can be measured in one single test.

REVIEW OF LITERATURE:

Parthasarathy Govindasamy[14] et al., (2013) prepared Buccal patches of carbamazepine with unidirectional drug release were HPMC, polyvinyl alcohol,polyvinyl yrrolidone,ethyl cellulose by solvent casting method.water impermeable layer of patches provide unidirectional drug release.They were evaluated for thickness,mass uniformity,surface pH,and floading endurance. The formulation were evaluated further for swelling studies,*exvivo*mucoadhesive strength,*exvivo* mucoadhesion time,*invitro* drug release,*exvivo* permeation, accelerated stability studies and FTIR and XRD spectral studies. The prepared unidirectional buccal patches of carbamazepine provided a maximum drug release within specified mucoadhesive period and it indicate a potential alternative drug delivery system for systemic delivery of carbamazepine.

Sarath chandran C [15] et al., (2013) investigated the ability of polymer to release the Bisoprolol fumarate in a controlled pre determined manner. The mucoadhesive buccal patches were prepared by solvent casting method with appropriate modification. The prepared patches were subjected to physical evaluations, *invitro* diffusion , and stability study. The result obtained was satisfactory with all the formulation, but, the patches prepared with 2% chitosan showed a better *invitro* diffusion result as it can diffuse 96% of drug within 12 h of the therapy. The result of physical evaluation and stability study indicating that the Bisoprolol buccal patches with 2% chitosan could effectively treat the possible anginal attack and hypertension.

Swati c. et[16] al.,(2013) showed that the solubility and dissolution rate of Ramipril were significantly improved by complexation with β-cyclodextrin and Hydroxy propyl

βcyclodextrin with respect to drug alone. The kneaded complex showed higher dissolution rate than other complex and it was incorporated into buccal Patches. The patches were prepared by solvent casting method using hydroxyl propyl methyl cellulose (HPMC K15) and Poloxomer. The patches were found to be smooth in appearance, uniform in thickness, weight uniformity, drug content, swelling index, folding endurance, surface pH and *invitro* diffusion study using Keshery chien diffusion cell. The Patch of 0.5% HPMC K15 exhibit *invitro* release of 81.16% through cellophane membrane and 75.72% release through egg membrane and the Patch of 0.5% Poloxomer exhibit *invitro* release of 77.24% through cellophane membrane and 71.08% release through egg membrane in 8 h showing good mucoadhesive strength and mucoadhesive time. The Optimized patch was subjected to *exvivo* studies through goat buccal mucosa showed 63.49% release in 8 h.

N.G. Raghavendra Rao and Keyur Patel[17]et al., (2013) developed and optimized the formulations of mucoadhesive patches of Ropinirole. Ropinirole buccal patches are prepared using different mucoadhesive polymers by solvent casting technique. Buccal patches were characterized for parameters like physical appearance and surface texture, mass uniformity, thickness, folding endurance, swelling index, surface pH, drug content uniformity, *invitro* residence time, Bursting strength, *exvivo* mucoadhesive force, *exvivo* permeation study, *invitro* drug release study and drug-excipients interaction study. Release of Ropinirole from all patches followed zero order and mechanism was diffusion rate limited. Ropinirole mucoadhesive buccal patches promising one as the controlled drug delivery, shows moderate swelling, convenient resident time will lead to improve the bioavailability and greater therapeutic efficacy.

Sellappan Velmurugan , P.Srinivas[18]et al., *(2013)* prepared mucoadhesive buccal tablets of Losartan potassium and evaluated for physicochemical parameters such as hardness,

thickness uniformity, weight variation, and surface pH and moisture absorption studies.*exvivo* mucoadhesive strength, *exvivo* residence time and *invitro* release studies showed that formulation containing 1:1.25 ratio of drug and polymer combination showed satisfactory bioadhesive and exhibited optimum drug release (91.33 % after 12h). The bioadhesive buccal tablets with desired permeability could be prepared. The Stability of Losartan potassium mucoadhesive buccal tablets was determined in natural human saliva; it was found that both Losartan potassium and buccal tablets were stable in human saliva.

Vandana Soni & Aviral Jai[19]et al., (2013) developed a controlled release drug delivery device of Glipizide to maintain its bioavailability over an extended period of time and to circumvent the hepatic first pass effect. Drugcoat and HPMC were used as a polymer for the preparation primary and secondary layer respectively, of controlled release bilayerd buccoadhesive patches of drug. The prepared patches were evaluated for various *invitro* and *invivo*studies. The developed bilayered buccoadhesive delivery system bears potential to deliver the drug in a controlled manner over an extended period of time.

Nagaveni Somepalli[20] et al.,(*2013)* prepared Salbutamol sulfate patches HPMC, SCMC and Carbopol 934 in various proportions and combinations using Glycerol and tween-80 as plasticizers. Patches were laminated on one side with a water impermeable backing layer using ethyl cellulose for unidirectional drug release. The thickness of medicated patches were ranged between 0.402 and 0.431 mm and mass varied between 0.0312 and 0.0352 g. The surface-pH of patches ranged between 6 and 7. All formulations showed good folding endurance. The maximum *invitro* release was found to be 93.89% over a period of 150 min. Data of *invitro* release from patches were fitted to different kinetic models such as Higuchi and Korsmeyer–Peppas models to explain the release profile.

B.Samyuktha Rani[21] et al., (2013) fabricated Venlafaxine HCL patches were by using sodium alginate with various polymers such as CMC, HPMC E15 in various proportions using solvent casting technique. Buccal patches were evaluated by different parameters such as thickness, weight uniformity, content uniformity, swelling index, surface pH, moisture up take study, moisture absorbance study, folding endurance and *invitro* drug release study and FTIR studies. The optimized fabricated patches showed that the drug release indicates non- fickian release kinetics and diffusion as chain relaxation mechanism. All the fabricated patches were effective and showed excellent sustained drug release for 8h.

J. Ravi Kumar Reddy[22] et al., (2013) fabricated Tapentadol hydrochloride buccal films by solvent casting technique with different polymer combinations of hydroxypropyl methylcellulose, chitosan, carbopol and carbopol. Drug–polymer interaction studies by Fourier transform infrared spectroscopy showed that there was no significant interaction between drug and polymers. Stability study of buccal films was performed in natural human saliva.*exvivo* permeation studies were conducted using fresh sheep buccal mucosa and buccoadhesive strength was calculated by modified balance method and showed sufficient strength in all the formulations. Good correlation was observed between the *invitro* drug release and *invivo*drug release, with a correlation coefficient of 0.996. Drug diffusion from buccal films showed apparently zero order kinetics and release mechanism was diffusion controlled after considerable swelling.

Rama devi Bhimavarapu [23]et al., (2013) formulated mucoadhesive buccal patches of sertraline hydrochloride inorder to bypass the first pass metabolism. The prepared patches were evaluated for their weight variation, thickness, folding endurance, surface pH, swelling index, moisture uptake study, moisture absorbance study, drug content uniformity and *in vitro* drug

release and FTIR studies were conducted for Drug – Excipient compatibility testing. The optimized patches showed that the drug release indicates non- fickian release kinetics and diffusion as chain relaxation mechanism. Formulation showed the highest release rate of 83.41% and all the prepared patches were effective and showed excellent sustained drug release.

Iswarya Sridhar[24] et al., (2013) optimized the formulations of mucoadhesive buccal patches of Ondansetron hydrochloride. The patches of all formulations showed good flexibility, mucoadhesive strength and smooth surface and were found to be stable. Drug content was found to be uniform in all the formulations and all the evaluation parameters were found to be within acceptable limits. The patches showed sustained release for a period of 8 h thereby achieving therapeutic efficacy and good patient compliance. The optimized patches were found to be stable when exposed to accelerated stability conditions.

Rama bukka[25]et al., (2012) formulated a buccal dosage form of Felodipine to increase its bioavailability by casting method using Polyethylene Oxide with hydroxy propyl cellulose (HPC) or Ethyl Cellulose using 23 factorial design. The solvent was ethanol and dichloromethane (1:1 ratio). The films were evaluated for *exvivo* mucoadhesive strength and *invitro* residence time, drug release and percentage swelling. The best set of formulation was F2, as per result of bioadhesive strength, *invitro* residence time which is having highest concentration of Polyethylene Oxide (0.1%) & HPC (2.5%). All the formulations were following the zero order release and non Fickian model of kinetic release.

M.Jyostna, Bhaskar Reddy[26] et al., (2012) prepared Buccal patches of Desloratidine by solvent evaporation method using HPMC 15 cps and xanthan gum which are the hydrophilic polymers in different ratios. The prepared patches were tested for physical parameters like

Thickness, Folding endurance, Uniformity of weight, swelling index and Surface pH of patches and *invitro* drug release studies. All the physical parameters fall within the limits. The drug content was uniform in all the formulated buccal patches of Desloratidine. The results indicate uniform distribution of drug within the patches. The release of Desloratidine from the buccal patch was sustained up to 6h.Among the five formulations, the F-V shows maximum drug release of 89.03% in 6 h. The optimized formulation follows zero order kinetics to release the drug from the patches.

N. G. Raghavendra Rao[27] et al., *(2012)* prepared buccal patches of zolmitriptan using hydroxy propyl methyl cellulose (HPMC). HPMC and gel forming polymers like HPMC (K4M), carbopol, poly vinyl pyrrolidone and Eudragit RL-100 by Solvent Casting technique. All the prepared patches were smooth surface and elegant texture. From among all the developed formulations, the formulation containing HPMC K4M and carbopol 934 retarded the drug release in a controlled manner for prolonged period of more than 8 h, gave satisfactory *invitro* resident time maximum duration of 7.46 h, so it was selected as the best formulation. Swelling studies indicated significant water uptake and contributed in drug release. Stability studies were as per ICH guidelines, suggesting that there was no significant change in surface pH, drug content, bioadhesion property, swelling behavior of the patches. FTIR studies revealed that, there was no incompatibility of the drug with the excipients used.

Jobin Jose[28] et al.,(2012) developed the mucoadhesive buccal patch of labetalol and systematically evaluate *invitro* performances of buccal films of labetalol hydrochloride by using chitosan, CARBOPOL K30, CARBOPOL K90, HPMC and polyethylene glycol (PEG) 1000 as

plasticizer. Buccal films were developed by solvent casting technique and all the formulations were examined for patch thickness, weight variation, drug content, surface pH, folding endurance, *invitro* residence time and *invitro* release. The all prepared buccal patches were transparent, smooth, consistent and flexible. The surface pH of all formulation showed to be neutral. It was found that formulations showed good swelling, a convenient residence time, as well as promising drug release pattern and the release of the drug from the patches followed the diffusion controlled mechanism in all the formulations.

Bharath Kumar.V[29]et al., (2011) prepared and evaluated mucoadhesive buccal films containing Diltiazem hydrochloride by employing HPMC, eudragit, ethyl cellulose alone and in combination with CARBOPOL. The prepared mucoadhesive buccal films showed uniform thickness, weight, folding endurance, surface pH, drug content and swelling index. The drug content of all the formulation was found to be uniform. *invitro* drug release studies indicated that the films prepared with HPMC (3%) and ethyl cellulose (4%) has shown fast and slow release respectively. The formulated films were stable during stability studies at 45ºC and 75%RH with respect to drug content.

Santosh Kumar[30] et al.,(2011) formulated mucoadhesive buccal patches of flurbiprofen (FBN) in order to enhance solubility. Solubity enhancement was attempted by making solid dispersion of drug with β-CD (cyclodextrin).. Buccal patches were prepared by solvent casting technique using polymers like polyvinyl alcohol (PVA), sodium carboxymethyl cellulose (SCMC), and hydroxypropyl methylcellulose (HPMC). The prepared patches were evaluated for their weight variation, thickness, folding endurance, surface pH, swelling index,*invitro* residence time,*invitro* permeation studies, drug content uniformity and bioadhesion test.

Marina Koland[31] et al., (2010) Buccal delivery is considered to be an important alternative to the peroral route for the systemic administration of drugs. Losartan potassium is an angiotensin II receptor antagonist with an oral bioavailability of only 33% due to extensive first pass metabolism. Mucoadhesive buccal films of losartan potassium were prepared using hydroxypropyl methyl cellulose (HPMC) and retardant polymers ethyl cellulose (EC) or eudragit RS 100. Films were flexible and those formulated from EC were smooth whereas those prepared from Eudragit were slightly rough in texture. The mucoadhesive force, swelling index, tensile strength and percentage elongation at break was higher for those formulations containing higher percentage of HPMC. *invitro* drug release studies reveal that all films exhibited sustained release in the range of 90.10 to 97.40 % for a period of 6 h. The data was subjected to kinetic analysis which indicated non fickian diffusion for all formulations except E2.*exvivo* permeation studies through porcine buccal mucosa indicate that films containing higher percentage of the mucoadhesive polymer HPMC showed slower permeation of the drug for 6-7 h.

Bingi Manasa[32] et al.,(2010) developed Resperidone patches using HPMC (15 & 47 cps), chitoson, poly vinyl alcohol, poly vinyl pyrilodine. The patches were evaluated for their thickness, Uniformity content, folding endurance, weight uniformity, Swelling index, tensile strength and surface pH.*invitro* loaded studies of resperidone-loaded patches in phosphate buffer(pH 6.6) exhibited drug release in the range of 67.32% to 98.28 in 60 min. Data of *invitro* release from patches were fit in to different equations and kinetic models to explain kinetics. The *invitro* release study showed that patches could deliver drug to the oral mucosa. The results indicate that the mucoadhesive buccal patches of resperidone may be good choice to bypass the extensive hepatic first pass metabolism with an improvement in the bioavailability of resperidone through buccal mucosa.

S.Velmurugan, B.Deepika[33] et al.,(2010) prepared Buccoadhesive tablets of piroxicam by using HPMC K4M and carbopol 934 as mucoadhesive polymers. The formulations were tested for *invitro* drug release, bioadhesive strength, moisture absorption, residence time and drug permeation through porcine buccal mucosa. Optimized formulation showed maximum release of the drug (97.67±0.41) with the peppas model release profile and permeated 26.52±0.19 of the drug through porcine buccal membrane. The suitable bioadhesive buccal tablets with desired permeability could be prepared. Stability of piroxicam buccal tablets was determined in natural human saliva; it was found that both piroxicam and buccal tablets were stable in human saliva.

Bhanja Satyabrata[34] et al.,(2010) designed and evaluated mucoadhesive bilayered buccal devices comprising of a methotrexate containing mucoadhesive layer and drug free backing membrane. sodium alginate alone or in combination with sodium carboxy methyl cellulose, polyvinyl pyrollidine and carbopol 934 and backing membrane (ethyl cellulose). The patches were fabricated by solvent casting technique and evaluated for film weight uniformity, thickness, swelling index, surface pH, mucoadhesive strength, mucoadhesive time and folding endurance, *invitro* and *exvivo* drug release. A combination of sodium alginate with carbopol 934 and glycerol as plasticizer gave promising results. The optimum patches exhibits an *invitro* release of 82% through cellophane membrane and 70.78% in 8 h through buccal mucosa with satisfactory, mucoadhesive strength mucoadhesive time. The *invitro* release kinetics through cellophane membrane fits well for Higuchi, while *exvivo* through buccal mucosa it followed zero-order kinetics. The *exvivo* data also fitted to Korsmeyer-Peppas equation which characterizes the release mechanism as non-Fickian, which means the drug release is independent of concentration gradients.

Satyabrata Bhanja[35] et al.,(2010) formulated and evaluated mucoadhesive buccal tablets of Timolol maleate to circumvent the first pass effect and to improve its bioavailability with reduction in dosing frequency and dose related side effects. The tablets were prepared by direct compression method. The best *invitro* drug release profile was achieved with the formulation contains the drug, Carbopol 934p and HPMC K4M in the ratio of 1:2.5:10. The *invitro* release kinetics studies reveal that all formulations fits well with zero order kinetics followed by Korsmeyer-Peppas, first order and then Higuchi's model and the mechanism of drug release is non-Fickian diffusion.

Subhash V. Deshmane[36] et al.,(2009) developed verapamil hydrochloride buccal patch to increase bioavailability and prevent first pass metabolism of drug The mucoadhesive strength, vapour transmission and *invitro* released of water soluble drug through water insoluble chitosan base matrix were found satisfactorily. The physical appearance of buccal patch was examined by scanning electron microscopy. The released kinetic model best to fit for the optimized batch was Hixson Crowell, indicating that the drug release from systems in which there is a change in the surface area and the diameter of particles present in dosage form.

***Gazzi Shankar[37] et al.,* (2009)[21]** formulated and evaluated bioadhesive buccal drug delivery of tizanidine hydrochloride tablets prepared by direct compression using bioadhesive polymers such as hydroxypropyl methyl cellulose K4M, sodium carboxymethyl cellulose and combination of these two polymers. In order to improve permeation of drug, the permeation enhancers like beta-cyclodextrin (β-CD), hydoxypropyl beta-cyclodextrin (HP-β-CD), and sodium deoxycholate (SDC) were added to the formulations. The β-CD and HP-β-CD were

taken in 1:1 molar ratio. *invitro* release of optimized batch was found to be non-Fickian. The stability studies in natural saliva indicated that optimized formulation has good stability in human saliva.

Deelip Derle[38]et al., **(2009)** formulated and evaluated buccoadhesive bi-layer tablet of propranolol hydrochloride using a buccoadhesive polymers such as sodium alginate and carbopol 971 P along with ethyl cellulose as an impermeable backing layer. Tablets containing sodium alginate and carbopol 971 P in the ratio of 5:1 showed the maximum percentage of *invitro* drug release without disintegration in 12 h. The swelling index was proportional to sodium alginate content and inversely proportional to carbopol 971 P content. The surface pH of all tablets was found to be satisfactory, close to neutral pH; hence, no irritation would observe with these tablets. The mechanism of drug release was found to be zero-order kinetics.

V.N. Deshmukh[39] et al., **(2009)** formulated and evaluated theophylline anhydrous bioadhesive tablets. Different types of natural hydrophilic polymers such as xanthium gum, locust gum, guar gum, karaya gum and their combinations were used to formulate matrix tablets. The combination of karaya gum:guar gum (6:4) tablet showed a greater boiadhesive strength as compared with a single gum and other gum combination tablets. Karaya gum:guar gum were not discharged from the mucus membrane and were dissolved in the gastric fluid. An increase in gum concentration increases the drug release profile beyond 12 h whereas there is no significant effect of gum concentration on the bioadhesive strength of the tablet.

Monica rao[40]et al., **(2009)** evaluated effervescent floating matrix tablet formulations of salbutamol sulphate. Two viscosity grades of HPMC as matrix materials were used for formulating the tablets, which are prepared by wet granulation. The *invitro* drug release

mechanism showed anomalous transport. An increase in polymer concentration and viscosity grade of the polymer resulted in a decrease in the release rate.

R Manivannan, A Balasubramaniam[41] et al.,(2008) fabricated Mucoadhesive buccal tablets of Diltiazem hydrochloride using carbopol-934, Sodium carboxy methyl cellulose (SCMC), Hydroxy propyl methyl cellulose (HPMC), sodium alginate and guar-gum as mucoadhesive polymers. The carbopol-934 is used as a primary polymer because of its excellent mucoadhesive property and secondary polymers like HPMC, SCMC, sodium alginate and guar-gum were used. The effect of secondary polymer loading on drug release was studied. Formulation follows zero order drug release. FTIR studies show no evidence on interaction between drug and polymers.

***R Manivannan[42] et al.,* (2008)** formulated and evaluated mucoadhesive buccal tablets of diltiazem hydrochloride using cabopol-934, sodium carbpxy methyl cellulose (SCMC), Hydroxy propyl methyl cellulose (HPMC), sodium alginate and guar-gum as mucoadhesive polymers. eight formulations were developed with varying concentration of polymers. The carbopol-934 is used as primary polymer because of its excellent mucoadhesive property and secondary polymers like HPMC, SCMC, sodium alginate and guar-gum were used. The effect of secondary polymers loading on drug release was studied. Formulations F2 showed maximum release of 76.98% in 8 h. Formulation F2 showed maximum swelling index of 3.7 after 8 h. Formulation FA2 follows zero order drug release. FTIR studies show no evidence on interaction between drug and polymers. The release indicates that suitable mucoadhesive buccal tablets with desired properties could be prepared.

Emami J [43] ***et al., (*****2008)** developed and evaluated controlled-release buccoadhesive verapamil hydrochloride tablets, to study controlled release buccoadhesive tablets inorder to achieve constant plasma concentrations, to improve the bioavailability by the avoidance of hepatic first-pass metabolism and to prevent frequent administration. The maximum bioadhesive strength was observed in tablets formulated with a combination of CP-NaCMC followed by CP-HPMC and NaCMC-HPMC. Decreasing the content of CP in CP-HPMC tablets or NaCMC in CP-NaCMC or NaCMC-HPMC systems resulted in decrease in detachment forces.

Ganesh P [44] ***et al.,*** **(2008)** developed and evaluated mucoadhesive buccal tablets of domperidone. The mucoadhesive polymers used in the formulations were Carbopol 934P, Methocel K4M, Methocel E15LV and Chitosan. Tablets were prepared by direct compression method using polymers in different ratios. The best mucoadhesive performance and *invitro* drug release profile were exhibited by the tablets containing chitosan and Methocel K4M in ratio of 1:1. It was observed that optimized batch follows Hixon Crowel release kinetics.

M. Nappinai, [45] ***et al.,(2008)*** formulated and evaluated nitrendepine buccal film using mucoadhesive polymers like HPMC K100, hydoxypropylcellulose, NaCMC, sodium alginate, polyvinyl alcohol, PVP K30 and Carbopol 934P. Based on the evaluation of these result it was concluded that buccal film made of hydroxypropylcellulose and NaCMC which showed moderate drug release and satisfactory film characterization could be selected as the best among formulation studies.

J.Ali [46] ***et al.,*****(2008)** prepared chronomodulated drug delivery of salbutamol sulphate for the treatment of nocturnal asthma. The core containing salbutamol sulphate were prepared by direct compression method using different ratios of microcrystalline cellulose and effervescent

agent and then coated sequentially with an inner swelling layer containing hydrocolloid, hydroxypropyl methyl cellulose and an outer rupturable layer having Eudragit RL/RS (1:1). The rupture and dissolution test were studied using the USP paddle method at 50 rpm in 0.1 N HCl and phosphate buffer pH 6.8. The lag time of drug release decreased by increasing the inner swelling layer and increased by increasing the rupturing layer level.

F.K. Alanazi1 [47]et al., (2007) ; formulated Ketorolac tromethamine buccoadhesive films to overcome the limitations in the currently available dosage and routes of administration which in sequence will increase patients compliance. Films were cast from organic and aqueous solvents using various bioadhesive polymers namely: sodium carboxymethyl cellulose (Na-CMC), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC) and Carbopol 934. Formulation containing carbopol 0.5% and HPMC 0.5% was found to be the best film as it shows good adhesion, acceptable pH, and gives a reasonable ketrolac release (about 85-90% at 6 h). In addition, this film was subjected to *Invitro* and *invivo* release. The obtained results indicate that the concentration of ketorolac in the oral cavity was maintained above 4.0 μg/mL for a period of at least 6 h. This film shows promising results for using the ketrolac buccoadhesive route of administration topically and systemically.

Vamshi Vishnu Yamsani[48] et al., **(2007)** developed and evaluated buccoadhesive carvedilol tablets, using HPMC K4M, HPMC K15M and carbopol 934 as mucoadhesive polymers. Formulations of the BC or BD series were composed of HPMC K4M or HPMC K15M in rations of 1:1 to 1:5 whereas in the BE series, Carbopol 934 was used (1:0.25 to 1:1.50). Formulation BC3 showed maximum release of the drug (88.7 ± 0.4%) with the Higuchi model release profile and permeated 21.5 ± 2.9% of the drug (flux 8.35 ± 0.291 μg h^{-1} cm^{-2}) permeation

coefficient 1.34 ± 0.05cm h^{-1}) through porcine buccal membrane. BC_3 formulation showed 1.62 ± 0.15 N of peak detachment force and 0.24 ± 0.11 ml of work of adhesion

P.D.Nakhat [49] ***et al.,*** **(2007)** studied on buccoadhesive tablets of terbutaline sulphate prepared by direct compression method using bioadhesive polymers like Caebopol 934P , Methocel K4M, Methocel K15M and sodium carboxy methyl cellulose either alone or in combination with baking layer of ethyl cellulose. The maximum boiadhesive strength was observed in tablets formulated with Carbopol 934P alone and strength decreases with decrease in its contents. The tablets were evaluated for *invitro* release in pH 6.8 phosphate buffer for 10 h using a standardized dissolution apparatus. In order to determine the mode of release, the data was subjected to Korsmeyer and Peppas diffusion model. All the formulations followed non-Fickian release mechanism. Carbopol 934P and methocel K4M in the ratio of 1:1 could be used to design effective and stable buccoadhesive tablets of tebutaline sulphate.

Pulak kumar metia [50] ***et al.,*** **(2007)** evaluated novel mucoadhesive buccal tablet of oxytocin were prepared as cores in adhesive cups with mucilage (DPM) isolated from edible Dispyros peregrine fruit. Core tablets were formulated with oxytocin using permeation enhancers, sodium taurocholate and sodium thioglycollate. *invitro* permeation studies of NMBTs were conducted in franz diffusion cell containing 50 ml phosphate buffer, pH 6.6, at 37±0.2^{0}C through bovine buccal mucosa.

M.V. Ramana [51] ***et al*** **(2007)** designed and evaluated mucoadhesive buccal drug delivery systems containing metoprolol tartrate. The mucoadhesive polymers used in this formulation were Carbopol 934, hydroxylpropylmethylcellulose, hydroxyethylcellulose and sodium carboxymethylcellulose. The formulations were characterized for physiochemical parameters,

invitro release studies and *in-vivo* placebo studies.The best mucoadhesive performance and *invitro* drug release profile were exhibited by the tablets containing hydroxyethylcelluslose and Carbopol 934 in ratio 1:2. This product was more comfortable to the user due to absence of erosion, faster hydration rate and less viscosity of surrounding environment. *In-vivo* placebo studies did not show any side effect.

Vishnu M. Patel[52]et al., **(2007)** developed and characterized, chitosan-containing mucoadhesive buccal patches of propranolol hydrochloride, using the solvent casting method and chitosan as a bioadhesive polymer and different ratios of chitosan to PVP K-30 were used. The patches were evaluated for their physical characteristics like mass variation, drug content uniformity, folding endurance*,exvivo* mucoadhesion strength,*exvivo* mucoadhesion time, surface pH, *invitro* drug release, and *invitro* buccal permeation study. Patches exhibited controlled release for a period of 7h. Incorporation of PVP K-30 generally enhanced the release rate. Swelling index was proportional to the concentration of PVP K-30. Optimized patches (F_4) showed satisfactory bioadhesive strength of 9.6 ± 2.0g, and *exvivo* mucoadhesion time of 272 minutes. The surface pH of all patches was between 5.7 and 6.3. Good correlation was observed between the *invitro* drug release and *invitro* drug permeation with a correlation coefficient of 0.9364.

Libero ltalo Giannola[53]et al., **(2007)** studied the release of naltrexone (NLX) on buccal mucosa Permeation inorder to access the aptitude of NLX to penetrate the mucosal barrier using Franz type diffusion cells and compared with data obtained by reconstituted human oral epithelium (100μm) thick. Tablets, designed fro Naltrexone hydrochloride (NLX) administration on buccal mucosa, were developed and prepared by direct compression of drug loaded (56%)

poly-octylcyanocrylate (poly-OCA) matrices. NLX is slowly discharged from buccal tablets following Higuchian kinetic.

Bhupinder singh[54]et al., **(2006)** formulated and optimized of controlled release mucoadhesive tablets of atenolol using response surface methodology using carbopol 934P and sodium CMC as polymers. Carbopol 934P and sodium carboxymethylcellulose were taken as the independent variables. Both the polymers had significant effect on the bioadhesive strength of the tablets measured as the force of detachment against porcine gastric mucosa ($P<0.001$), the study helped in finding the optimum formulation with excellent bioadhesive strength and controlled release. Compressed matrices exhibited non-Fickian drug release kinetics approaching zero-order, as the value of release rate exponent (n) varied between 0.6672 and 0.8646, resulting in regulated and complete release until 24 h.

R.C.Doijad[55]et al., **(2006)** formulated and evaluated buccoadhesive drug delivery system of isosorbide dinitrate for improving bioavailability using solvent casting method and different bioadhesive polymess like carbopol 934P and carbopol using two different plasticizers PEG and diethyl phthalate. Unidirectional release was achieved by preparing composite films with backing membrane. i*nvitro* studies revealed that release rate of isosorbide dinitrate was higher from carbopol films containing ratio of Eudragit RL 100 and carbopol in proportion of 1:2 and 2:1, respectively by using both plasticizers. Drug diffusion from buccal films showed apparently zero order kinetics and release mechanism was diffusion controlled after considerable swelling.

Brunella Cappello[56]et al., **(2006)** developed cyclodextrin- containing poly (ethyleneoxide) tablets for the delivery of poorly soluble drug carvedilol (CAR) using PEO as bioadhesive SR platform and HPβCD as modulator of drug release. When the drug was incorporated as CAR/HPβCD freeze-dried product, all CAR content was released from the tablet in about 10 h, displaying a constant release regimen after a transient. The effect of HPβCD

incorporation of the release mechanism was rationalized on the basis of the interplay of different physical phenomena: erosion and swelling of the tablet, drug dissolution, drug counter-diffusion and complex formation.

***N.Venkatesan[57]et al.,*(2006)** studied on pharmacokinetic and pharmacodynamic following oral administration of erythropoietin mucoadhesive tablets to beagle dogs using an absorption enhancer labrasol in rats and dogs. Tablets were prepared using sylysia 550 (Porous Silica oxide) holding the absorption enhancer and carbopol 974 P as a muccoadhesive agent covered with a water insoluble backing layer of cellulose acetate and a pH sensitive covering layer of Eudragit L/Eudragit S.

***Shaila Lewis[58]et al.,*(2006)** designed, evaluated and studied pharmacokinetic study of mucoadhesive buccal tablets of nicotine for smoking cessation. Three types of tablets were developed each containing two mucoadhesive components (HPMC K4M and sodium alginate), (HPMC K4M and Carbopol), (Chitosan and sodium alginate). For each of these types, batches were produced changing the quantity of polymers resulting in nine different formulations. Pharmacokinetics studies were conducted in smokers. A peak plasma concentration of 16.78±2.27 ng was obtained in 2 h. which suggest potential clinical utility in nicotine replacement therapy.

***Kasshapa Goud H.Desai[59]et al.,* (2004)** prepared and evaluated, a novel buccal adhesive system, containing propranolol hydrochloride using special fabricated punch and partitioning medium as a phosphate buffer solution pH 6.6 and L-octanol and permeability coefficient through porcine buccal mucosa. NBAS was evaluated by weight uniformity, thickness, hardness, friability, swelling, mucoadhesive strength, *invitro* drug release, and *invivo* human acceptability studies. Swelling index was higher (4.4) for formulations containing hydroxy propyl methyl

cellulose (HPMC) K4M alone, and it decreases with its decreasing concentration in the NBAS. All NBASs showed higher MS with procine buccal mucosa when compared with that of rabbit buccal mucosa. The mechanism of pH release was found to be by non-Fickian diffusion and followed first order kinetics.

Solimon Mohammadi-Samani[60]et al.,(2004) formulated and evaluated prednisolone buccoadhesive tablets. The effect of mucoadhesive polymers such as HPMC with viscosity grade 60 and 500 mpas, NaCMC and Cp 934 alone or in combination with each other on the release profile of prdnisolone was studied and mucoadhesion strength of these buccoadhesive formulations was evaluated. The release of prednisolone from HPMC with viscosity grade 60 mpas and Cp 934 alone was fast and there mucoadhesion strength was low. On the other hand, the release rate of prednisolone from the HPMC viscosity grade 500 mpas and NaCMC and mucoadhesion strength were moderate and suitable. The result showed that with different blend of HPMC viscosity grade 500 mpas or NaCMC and Cp 934 with increasing in HPMC or NaCMC / Cp 934 ratio a remarkable decrease in the rate of drug release and appreciable increase in the mucoadhesion strength was observed. Except from the formulation prepared with HPMC viscosity grade 60 and 500 mpas other formulation has more fluctuation in release profiles and their kinetics of release was not fitted to zero order model.

Luana Perioli[61]et al., **(2004)** developed mucoadhesive patches for buccal administration of ibuprofen using several film forming and mucoadhesive polymers. The best film containing PVP as film forming and NaCMC as mucoadhesive polymer was loaded with ibuprofen and *invitro* and *invivo* release studies performed. Statistical investigations of *invitro* drug release showed that the diffusion was main drug release mechanism and Higuchi's model provided the best fit.

Mario Jug[62]et al., **(2003)** studied on influence of hydroxypropyl-β-Cyclodextrin complexation on piroxicam release from buccoadhesive tablets solubility studies included the formation PX (Piroxicam) and (HPβCD) (Hydroxypropyl-β-cyclodextrin) inclusion complex with 1:1 stoichiometry prepared and characterized by DSC, FTIR and X-RD. Tablets were prepared by direct compression of hydroxpropylmethyl cellulose (HPMC) and Carbopol 940 (C940), which showed superior bioadhesion properties compared to HPMC. The *invitro* release results demonstrated that matrix tablets containing the PX- (HPβCD) solid complex displayed faster PX release compared to those containing a physical mixture of "free" drug.

Luana Perioli[63]et al., **(2003)** studied on novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease. Mucoadhesive tablets were developed using different mixtures of cellulose derivates (Hydroxypropyl cellulose, Hydroxy ethyl cellulose, HPMC K4M, HPMC K15M, Sodium carboxymethyl cellulose) and polyacrylic derivatives (Carbomer 940, Carbomer 971 and polycarbophil). The best mucoadhesive performance and *invitro* drug release profile were achieved using hydroxyethylcellulose and carbomer 940 2:2 ratio.

J.Varshosaz[64]et al., **(2002)** developed and characterized buccoadhesive nifedipine tablets by direct compression of CMC with carbomer which showed superior bioadhesion properties compared to PVP, PVA, HPMC and acacia. The tablets containing 30 mg of nifedipine and various amounts of CMC and CP showed a zero-order drug release kinetic. The adhesion force was significantly affected by the mixing ratio of CP:CMC in the tablets. The weakest and highest adhesion force was observed at the mixing ratios of 1:0 and 8:2 of CP: CMC, respectively. The tablets containing 15% CMC and 35% CP adhered for over 8 h to the upper gums of six healthy

human volunteers. These tablets released about 56% of the loaded drug after 8 h *invivo* with a rate of 2.17 h^{-1} and were perfectly tolerated.

***Juan Manuel Llabot[65]et al* .,(2002)** formulated double layered mucoadhesive tablets containing nystatin. Lactose CD (direct compression), carbomer (CB), and hydroxyl propyl methyl celluslose (HPMC) were used as excipients. The immediate release layer was made of lactose CD (100 mg) and nystatin (3o mg). The CB:HPMC 9:1 mixture showed the best mucoadhesion properties and was selected as excipient for the mucoadhesive polymeric layer (200 mg). The incorporation of nystatin (33.3 mg) in this layer affected the water uptake, which in turn modified the erosion behavior. Nystatin showed first-order release. The tablet releases nystatin quickly from lactose layer and then in a sustained way, during approximately 6 h , from the polymeric layer. The mixture CB: HPMC 9:1 showed good mucoadhesion. A swelling diffusion process modulates the release of nystatin from this layer. A non-Fickian (anomalous) kinetic was observed.

***Chong-Kook Kim[66]et al.,* (2000)** formulated and evaluated omeprazole buccal adhesive tablet composed of sodium alginate, hydroxyl propyl methyl cellulose (HPMC), magnesium oxide and crosscarmellose sodium. Crosscarmellose sodium enhanced the release of omeprazole from the tablets. The analysis of the release mechanism showed that croscarmellose sodium changed the release profile of omeprazole from first- to zero-order release kinetics by forming porous channels in the matrix tablet matrix. However, it decreased the bioadhesive forces and stability of omeprazole tablets in human saliva. The tablet is composed of omeprazole-sodium alginate-HPMC-magnesium oxide-croscarmellose sodium (20:24:6:50:10). The plasma concentration of omeprazole in hamsters increased to reach a maximum of 370 ng/ml at 45 min after buccal administration and remained at the high level of 146-366 ng/ml for 6 h. The buccal

bioavailability of omeprazole in hamsters was 13.7 ± 3.2 %. These results demonstrate that omeprazole buccal adhesive tablets would be useful to deliver omeprazole which degrades very rapidly in acidic aqueous medium and undergoes hepatic first- pass metabolism after oral administration.

Yvonne T.F.Tan[67]et al., **(2000)** studied the effect of carbopol and polyvinylpyrrolidone on the mechanical, rheological, and release properties of bioadhesive polyethylene glycol gels: examining the properties of PEG gels that contains different ratios of carbopol 934P and PVP K90 using texture analyzer (TA-XT2) and rheometer (Rheomat 115A). In addition, lidocaine release from gels was evaluated using a release apparatus simulating the buccal condition. The results indicated that an increase in CP concentration significantly increased gel compressibility, hardness, and adhesiveness, factors that affect ease of gel removal from container, ease of gel application onto mucosal membrane, and gel bioadhesion. All PEG gels exhibited pseudoplastic flow with thixotropy, indicating a general loss of consistency with increased shearing stress. Drug release $T_{50\%}$ was affected by the flow rate of the simulated saliva solution. A reduction in the flow rate caused a slower drug release and hence a higher $T_{50\%}$ value.

Deepak Tiwari[68] et al., **(1999)** evaluated polyoxyethylene homopolymers for buccal bioadhesive drug delivery device formulation using instrona tensile tester with glass plate and bovine sublingual tissue as substrate surfaces. Several BBD device formulations containing polyoxyethylene polymer were prepared by direct compression and compression molding processes. The bioadhesive strength of polyoxyethylene polymers appeared to be directly related to their molecular weights. The drug release and the bioadhesive strength of the similarly prepared device formulations appeared to be dependent on the drug:polymer ratios. The elasticity

of the BBD devices prepared by compression molding was improved by the inclusion of polyisobuylene polymer in the formulations.

Rajesh Khanna[69]et al., **(1996)** prepared and evaluated bioerodible buccal tablets containing clotrimazole, for local delivery of clotrimazole to the oral cavity using different bioadhesive polymers along with soluble excipients like mannitol and PEG 6000. The *invitro* adhesion time and release characteristics were found to be a function of the type of polymer and also the total composition of the tablets. *invitro* evaluation of placebo tablets in healthy human volunteers indicated a linear and positive correlation between the *invitro* and *invivo* adhesion time.

Taina Sirkia[70] et al., **(1993)** developed press-coated prolonged-release salbutamol sulphate tablet were prepared using a compression-coating technique. Salbutamol sulphate was divided between the core and the coat in the ratio 2:1 or 1:2. Different viscosity grades and amounts of HPMC were used in the coat. When HPMC K100 was used, the release of salbutamol sulphate from tablets with 2/3 of the drug in the core increased with time. The release patterns obtained in the 1/3 of the drug in the core were biphasic. With other HPMC grades, the release patterns were best described by zero-order kinetics with 2/3 of the drug in the core and square-root-of-time kinetics with1/3 of the drug core in the core. For all formulations, an increase in the amount of HPMC decreased drug release.

3. RESEARCH INVESTIGATION

3.1. AIM OF THE WORK

Over the last two decades mucoadhesion has become of interest for its potential to optimize localized drug delivery, by retaining a dosage form at the site of action (e.g. within gastrointestinal tract) or systemic delivery, by retaining a formulation in intimate contact with the absorption site (e.g. the buccal cavity).

The present study indicates a good potential of erodible mucoadhesive buccal films containing Hydrochlorothiazide for systemic delivery with an added advantage of circumventing the hepatic first pass metabolism and immediate release. Buccal mucosa is rich in blood supply which acts as a perfect and fast site for absorption of drug.

Hydrochlorothiazide is a diuretic drug, frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones. Adverse effects of Hydrochlorothiazide are High blood sugar, Hyperlipidemia, Nausea/vomiting, Weight gain, Gout and Pancreatitis.

The aim of the present study was to prepare and evaluate buccal films containing Hydrochlorothiazide with the following objectives.

- To reduce the first pass metabolism
- To overcome the bioavailability problem.
- To improve the patient compliance.

3.2. PLAN OF WORK

The present work was carried out to prepare and evaluate buccal films of hydrochlorothiazide, as follows.

- Preparation of buccal films using various combination of different polymers.
- Evaluation of buccal film for the following physical and microbiological parameters.
    - Percent moisture absorption
    - Percent moisture loss.
    - Thickness
    - Uniformity of drug content.
    - Microbial count.
    - *Invitro* evaluation of buccal film.
    - *Exvivo* evaluation of buccal film in Goat buccal membrane.
- Stability studies
- Drug release kinetic studies.
- *Invivo* evaluation of buccal film in rabbits.

3.3. SUITABILITY OF HYDROCHLOROTHIAZIDE FOR BUCCAL DELIVERY SYSTEM

As it is mentioned in the introduction part, the choice of drug should be given careful consideration. Suitability of hydrochlorothiazide for various important criteria should be considered as buccal delivery.

Criteria 1: DRUGS WITH MOLECULUR WEIGHT

Molecular weight of Hydrochlorothiazide is 297.74.

Criteria 2: DRUG WITH LOW MELTING POINT

Melting point of Hydrochlorothiazide is 273 to 275 $^{\circ}C$

Criteria 3: DRUG ABSORTION AND FATE

14C – Hydrochlorothiazide was administered orally (n=4) and Iv (n=2) to healthy subjects. The gastrointestinal absorption ranged between 60% and 80% most of it took place in the duodenum and the upper jejunum. The plasma levels of HCT detuned according to 2 – compartment model,the half lives of the alpha and beta phases being 1.73 and 13.1 h respectively.Hydrochlorothiazide accumulated in the blood cells and the ratio between the radioactivity in cells and that in plasma averaged 3.5,the fate of a single daze of 14C – hydrochlorothiazide, in 2 hypertensive patient treated within the drug chonically was similar to that in the healthy subjects. A third person who had slightly elevated serum, creatinine,eliminated. Hydrochlorothiazide more slowly than the others. Like the healthy subjects, patient eliminated hydrochlorothiazide to greater than 95% in unchanged form.

4. MATHODOLOGY

4.1. MATERIALS USED

Table No: 1

S.NO	MATERIALS	SUPPLIER
1	Hydrochlorothiazide	Hyrodiuril, par pharmaceutical company,chennai
2	Hydroxy Ethyl cellulose	Microlabs,Hosur
3	Carbopol	Microlabs,Hosur
4	Hydroxy propyl methyl cellulose	Microlabs,Hosur
5	Polyvinyl alcohol	Bombay Lubricants Oil Company, Mumbai
6	Choloroform.	S.D. Fine Chemicals, Boisar
7	Potassium di hydrogen phosphate	Meryer Chemical Technology Co.,Ltd., Shanghai.
8	Sodium hydroxide	Indenta Chemical Pvt.Ltd. Mumbai India
9	Methanol	S.D. Fine Chemicals, Boisar
10	Acetone	S.D. Fine Chemicals, Boisar
11	Glycerol	S.D. Fine Chemicals, Boisar
12	Dimethyl sulfoxide	Gaylord Chemical Home,USA.

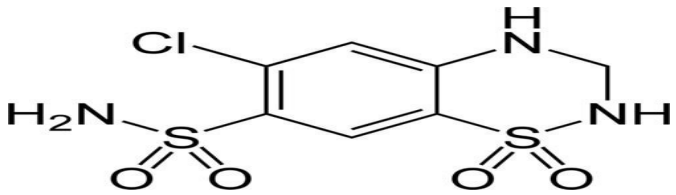
4.2. INSTRUMENTS USED

Table No:2

S.NO	EQUIPMENTS	SUPPLIER
1	Vortex mixture(G560)	Remi motors Ltd, Mumbai
2	Centrifuge(KW60)	Sharplex Filter Pvt.Ltd., Mumbai
3	Dessicator(A950)	Edutek Instrumentation, Ambala.
4	Petridishes	Sunshine Instruments, Coimbatore
5	Mini operation table(340294)	Medi World,Chennai
6	Magnetic Stirrer(IKA)	Remi motors Ltd, Mumbai
7	Double beam UV spectro photometer(UVD2950)	Perkin Elmer,Germay.
8	Electronic balance(C151)	Swastik ,New Delhi
9	pH-meter(215)	Elico Pvt Ltd,Chennai
10	Incubator(030712)	M.C.DALAL&CO, Mumbai
11	Freeze Drier(75035)	Allied,Mumbai.

DRUG PROFILE[71]

HYDROCHLOROTHIAZIDE:



Molecular weight = 297.74

Category **:** Inhibitors of Na^+, Cl^- (Diuretic , congestive heart failure)

Description **:** White crystalline powder.

Molecular formula **:** C_7H_8 Cl N_3 O_4 S_2

Solubility :

- Soluble in NaOH , Butyl amine, Dimethyl formamide, Acetone.
- Very slightly soluble in water
- Sparingly soluble in methanol
- Sparingly in soluble in ether $CHCL_3$,mineral acids.

Standards :

Hydrochlorothiazide is 2H – 1,2,4,benzothiazine- 7-sulfonamide 6cholro,3,4dihydro 1,1dioxide. It contains not less than 98% and not more than 102% of C_7H_8 Cl N_3 O_4 S_2 calculated with reference to the dried substance.

Storage :

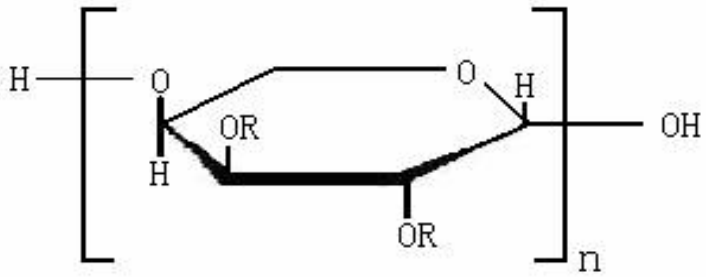
Preserve in well closed container.

4.3. POLYMER PROFILE

4.3.1. HYDROXY PROPYL METHYL CELLULOSE[72]

Molecular weight : 10,000-1,50,000

STRUCTURE:



SOLUBILITY

Soluble in cold water, certain grades of HPMC are soluble in aqueous acetone solution, mixture of dichloro methane and propen-2-ol and other organic solvents.

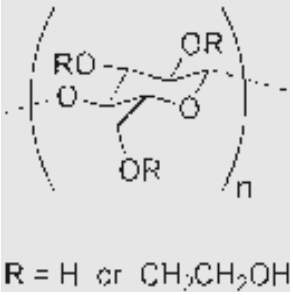
FUNCTIONAL CATEGORY

Films former , binder (2-5%), Thickening agent (0.45-1%).

STABILITY AND STORAGE CONDITION

Very stable in dry condition solution staple in pH 3.0 to pH11.0 .

4.3.2. HYDROXY ETHYL CELLULOSE[73]



Chemical name : Cellulose , 2 –Hydroxy ethyl ether.

FUNCTIONAL CATEGORY

Coating agent, suspending agent, tablet binder , thickening agent, viscosity increasing agent.

DESCRIPTION

Hydroxy ethyl cellulose occurs as a Light tan (or) cream to white coloured , odourless and tasteless, hygroscopic powder .

MELTING POINT

Soften at 135°C-140^{0}C decompose at about 205^{0}C.

MOISTURE CONTENT

Commercially available grades of Hydroxy ethyl cellulose contains less than 5% w/w of water.

SOLUBILITY

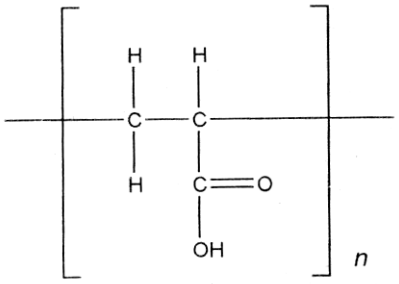
Hydroxy ethyl cellulose is soluble in either hot (or) cold water, forming clear, smooth, uniform solution practically insoluble in acetone,ethanol, ether, toluene and most other organic solvents.

APPLICATION IN PHARMACEUTICAL FORMULATIONS

Hydroxy ethyl cellulose is used as a thickening agent in ophthalmic and topical formulation , although it is also used as a binder and films coating for tablets.

4.3.3. CARBOPOL 934P[74]

Structural formula:



Nonproprietary Names BP : Carbomers

PhEur : Carbomera

USPNF : Carbomer

Synonyms Acritamer; acrylic acid polymer; Carbopol; carboxy polymethylene, polyacrylic acid; carboxyvinyl polymer; Permulen; Ultrez.

Chemical Name Carbomer [9003 – 01 – 4]

Carbomer 910, 934, 934 P, 940, 941, 971 P and 974 P resins.

Empirical formula Carbomers are synthetic high-molecular weight polymer of acryclic acid that are cross linked with either allylsucrose or allylethers of pentaerythitol. They contain between 56% and 68% of carboxylic acid (COOH) groups calculated on dry basis.

Molecular Weight 7×10^5 to 4×10^9

Functional Category Bioadhesive, emulsifying agent, release modifying agent, suspending agent, tablet binder, viscosity - increasing agent.

Description Carbomers are white-coloured, mildly acidic, hygroscopic powders with slight characteristic odour.

pH 2.7 – 3.5 for a 0.5% w/v aqueous dispersion 2.5 -3.0 for

	a 1% w/v aqueous Dispersion
Glass transition temperature	100-105ºC
Melting point	Decomposition occurs within 30 min at 260^0C
Moisture content	2.0% w/w maximum
Equilibrium moisture content	8 – 10 % w/v (at 50 RH)
pKa	6.0 ± 0.5
Equivalent weight	76 ± 4
Specific gravity	1.41
Density bulk	1.76 -2.08 g/cm^3
Density tapped	1.4 g/cm^3
Viscosity	29,400– 39,400 of 0.5% w/v solution.
Stability and storage conditions	Carbomers are stable, hygroscopic material that may be heated at temperatures below 104^0C up to 2 h. Without affecting their thickening efficiency. However, exposure to excessive temperature can result in discolouration and reduced stability. Carbomer powder should be stored in an air tight, corrosion-resistant container in a cool and dry place. The use of glass, plastic, or resin –lined containers is recommended.
Incompatibilities	Carbomers are discoloured by resorcinol and are incompatible with phenol, cationic polymer, strong acid and high levels of electrolytes.
Safety	Non - toxic and non –irritant

Applications	In liquid or semi solid pharmaceutical formulations - as suspending or viscosity- increasing agent, In tablets - as binders and as rate controlling excipient and as bioadhesive material, In emulsions - as emulsifying agent.

5. EXPERIMENTAL INVESTIGATIONS

5.1. STANDARD CURVE FOR HYDROCHLOROTHIAZIDE[75]

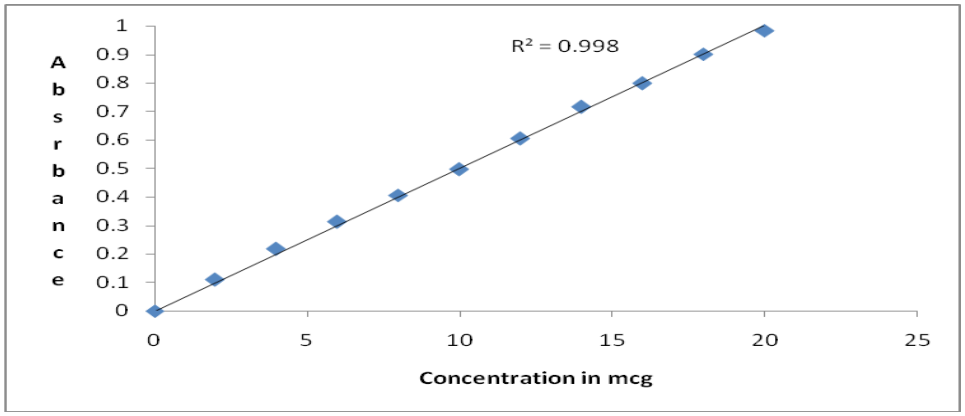
PROCEDURE

Weigh 100 mg of hydrochlorothiazide accurately and dissolved in little amount of phosphate buffer (pH6.8)[76] and the volume is made up with phosphate buffer to 100ml in a volumetric flask, from this 2ml was pipette out & transfer in to 100ml volumetric flask, and the volume is made up with phosphate buffer(pH6.8). From the above1ml was taken and made up to10ml with phosphate buffer(pH6.8). The intensity of the resulting solution was measured at 274nm against reagent blank adjusted to zero value. Beer's law obeyed in this range of 2-20µg/ml.

TABLE 3: **STANDARD CURVE OF HYDROCHLOROTHIAZIDE**

S.NO	CONCENTRATION (mcg)	ABSORBANCE
1	2	0.11
2	4	0.218
3	6	0.313
4	8	0.406
5	10	0.497
6	12	0.604
7	14	0.716
8	16	0.798
9	18	0.902
10	20	0.984

FIG 5: **STANDARD CURVE OF HYDROCHLOROTHIAZIDE**



5.2. FABRICATION OF BUCCAL FILMS

Films were prepared by the method of casting on glass plate.

FABRICATION OF DRUG RESERVOIR FILMS

Requited amount of HPMC or HEC was dissolved in water, the previously mixed HPMC solution, to this Glycerin 30% was included and poured over the glass substrate to get dry film.

FABRICATION OF DRUG RESERVOIR FILM WITH CARBOPOL:

Required amount of carbopol was dissolved in water,The previously mixed carbopol solution was added to the drug ,to this glycerin 10% was included and poured over the glass substrate to get a dry film.

COMPOSITION OF HYDROCHLOROTHIAZIDE BUCCAL FILMS

Table: 4

Composition	B1	B2	B3	B4	B5	B6	B7	**B8**	B9
HPMC	3%	4%	5%	4%	4%	4%	4%	**4%**	4%
HEC	-	-	-	2%	3%	4%	3%	**3%**	3%
CARBOPOL	-	-	-	-	-	-	1%	**2%**	3%
GLYCERINE	30%	30%	30%	30%	30%	30%	30%	**30%**	30%

Each film contain 25mg in drug

Concentration of plasticizer- 30%

5.3. EVALUATION OF PHYSICAL AND MICROBIOLOGICAL CHARATERSICS OF BUCCAL FLIMS

5.3.1. PERCENT MOISTURE ABSORPTION

The percent moisture absorption test was carried out to check the physical stability of the buccal films at high humid condition. In the present study the moisture absorption capacity of the films were determined as follows.

The 1.00cm diameter films were cut out and weighed accurately then the films were placed in desiccators containing saturated solution of aluminium choloride, keeping the humidity inside the desiccators 79.5%, after thee days, the films were removed, weighed and percentage moisture absorption was calculated. Average percentage moisture absorption of the films was found.

$$\text{Percent moisture absorption} = \frac{final weight - initial weight}{initial weight} \times 100$$

5.3.2. PERCENTAGE MOISTURE LOSS

This test was carried to check the integrity of films at dry condition. The 1.00cm diameter films was cut out and weighed accurately and kept in a dessicator containing fused anhydrous calcium choloride. After 72 h the films were removed and weighed. Average percentage moisture loss of thee films were found out.

$$\text{P.M.L} = \frac{Initial weight - final weight}{Initial weight} \times 100$$

5.3.3. SWELLING INDEX

The films are taken from each batch and it placed in pH6.8 phosphate buffer and the weight is measured every three minutes, untill the weight becomes constant.

5.3.4. WATER VAPOUR TRANSMISSION RATE

For water vapour transmission rate, glass vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in oven. About 1 gm of anhydrous calcium chloride was taken in the cells and the polymer films was fixed over the brim with the help of the solvent. Cells were accurately weighed, kept in a closed desiccators containing saturated solution of potassium to maintain a humidity of chloride 84%RH. The cells were taken and weighed after 6,12,24,36,48,72h of storage. The amount of water vapour transmission was found using the formula.

$$\text{Water vapour transmission} = \frac{\text{final weight} - \text{initial weight}}{\text{time} \times \text{area}}$$

Water vapour transmission rate is usually expressed as the number of grams of moisture gms/h/sq.cm. from the data obtained water vapour transmission was calculated.

5.3.5. THICKNESS

Thickness of the films were measured at six different points using a screw guage and average thickness of thee films were found out.

5.3.6. WEIGHT OF BUCCAL FILMS

Each films was weighed individually and average weight of the films were found out.

5.3.7. FOLDING ENDURANCE

It was determined repeatedly by folding a small strip of films at the same place till it broke. The number of times, the films could be folded at the same place without breaking is the value folding endurance.

5.3.8. DRUG CONTENT

A film size of 1cm diameter was cut and dissolved in phosphate buffer(pH6.8). After adding suitable reagent and dilution, optical density was found out at 274nm. Average drug content of thee buccal films were determined.

5.3.9.MEASUREMNT OF BIOADHESIVE STRENGTH OF MUCOADHESIVE DRUG RESERVOIR FILMS

Mucoadhesive strength of tablet was measured with porcine buccal mucosa using a modified 2-arm balance. The design of apparatus used while measuring the mucoadhesive strength is shown in **FIG.6**. Porcine buccal mucosa was obtained from a local slaughter house and stored in phosphate buffer pH 6.8 upon collection. The experiments were performed within 3

h of procurement of the mucosa. The porcine buccal mucosa was fixed to the stainless steel piece with cynoacrylate adhesive and then placed in a beaker. Phosphate buffer pH 6.8 was added into the beaker up to the upper surface of the buccal mucosa to maintain buccal mucosal viability during the experiments. The film was attached to the upper clamp of the apparatus and then the beaker was raised slowly until contact between porcine buccal mucosa and film was established. A preload of 50 g was placed on the clamp for 5 minutes (preload time) to establish adhesion bonding between tablet and porcine buccal mucosa. The preload and preload time were kept constant for all the formulation. After completion of the preload time, preload was removed from the clamp and water was then added in the beaker from the burette at a constant rate of 100 drops per minute. The addition of water was stopped when film was detached from porcine buccal mucosa. The weight of water required to detach film from buccal mucosa was noted as mucoadhesive Strength, and these experiments were repeated with fresh mucosa in an identical manner.

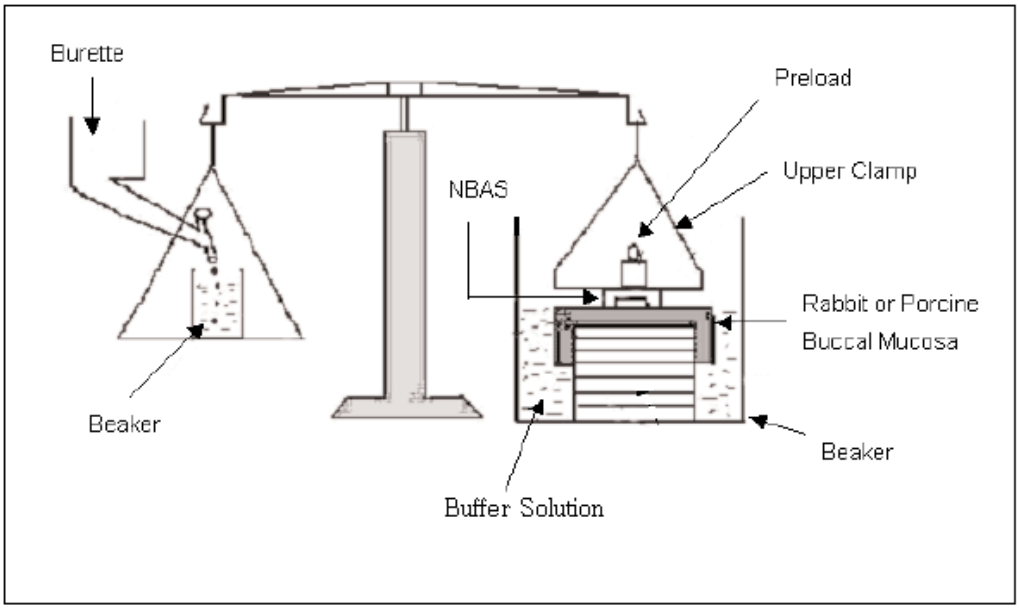


FIG 6: Illustration of modified apparatus for *invitro* mucoadhesive strength.

5.3.10. MICROBIAL COUNT

The film strip of different formulation were cut into small circular strips at 1.0 cm in diameter and aseptically transferred on to a nutrient agar in pertridishes and was moistened by the addition of 1ml of sterile distilled water. Control plates were also maintained where only 1 ml of sterile distilled water was added. The plates were examined for microbial growth after 24 h of incubation.

5.4. *INVITRO* DRUG RELEASE STUDIES OF HYDROCHLOROTHIAZIDE BUCCAL FILMS

Commercial semi permeable membrane was employed in this study. The membrane used was transparent and regenerated cellulose type which was permeable to low molecular weight substances.

A film size of 1 cm diameter was cut and placed on the semi permeable membrane. The semi permeable membrane was tied to one end of an open end cylinder, which act as donor compartment. The entire surface of the membrane was in contact with receptor compartment containing 100 ml of phosphate buffer (pH = 6.8). The content of the compartment was agitated by a magnetic stirrer.

Sample of 1 ml were withdrawn from receptor compartment and replaced by equal volumes of fresh media. The withdrawn samples were analysed using UV spectro photometer at 274 nm using reagent blank.

5.5. *INVIVO* DRUG RELEASE STUDIES[77]

METHODS

A healthy rabbit weighing 2.5 to 3kg was taken which was already checked for absence of any diseases. The fore limbs and hind limbs were tied into the iron of the mini operation table; so that rabbit was in dorsal position. The prepared film having the size of 1cm containing 20 mg of hydrochlorothiazide was placed in buccal memberane with the help of clip. Dextrose solution was transfused continuously thoughout the period of study. Periodically 1ml blood samples were taken using a syringe which already contained 1ml of 3.8% sodium citrate solution to prevent blood clotting. These blood samples were subjected for centrifuging at 2,200 rpm for about 20minutes. 1ml of supernatent liquid was taken from this and after suitable dilution these samples were analyzed at 274nm using spectrophotometer.

5.6. STABILITY STUDIES[78]

The optimized formulation was subjected to accelerated stability storage conditions for 3 months stored at 4^0C, Room temperature and 40^0C/75% RH in Stability Chamber. At the interval of one month intervals buccal films were withdrawn and evaluated for various physical parameters, and *invitro* drug release.

6. RESULTS AND DISCUSSION

In the present study the buccal drug delivery system of Hydrochlorothiazide were prepared by using different hydrophilic polymer such as HPMC, HEC, Carbopol K-30 by solvent evaporation technique.

6.1. PREFORMULATION STUDIES

1) ORGANOLEPTIC EVALUATION OF PURE DRUG

Hydrochlorothiazide is white or almost white crystalline powder and odorless.

2) BULK CHARACTERIZATION

2.1. Moisture Content : Moisture content of Hydrochlorothiazide was found to be 0.17%.

2.2. Melting Point : The melting point of Hydrochlorothiazide determined by open capillary method and was found to be 273-275^{o}C

2.3. Solubility : Hydrochlorothiazide soluble in ethanol, poorly soluble in water.

6.2. DRUG-EXCIPIENTS COMPATIBILITY

According to the physical Drug-Excipients compatibility study it was found that the polymer as well as the excipients selected was compatible with the drug Hydrochlorothiazide. So the excipients and polymers were selected for formulation.

Table 5: Drug and Excipients Compatibility.

Sl. No.	Drug :Excipients	Ratio	Initial physical observation	Condition (40^{0}C/75%RH) After 15 days
1	Drug	1:0	White crystalline powder	No change
2	Drug:HPMC	1:1	White crystalline powder	No change
3	Drug:HEC	1:1	White crystalline powder	No change
4	Drug:CARBOPOL	1:1	White crystalline	No change

6.3. FT-IR STUDIES:

The interpretation of FT-IR spectrum of pure drug, polymers and optimized formulation is shown in table 5

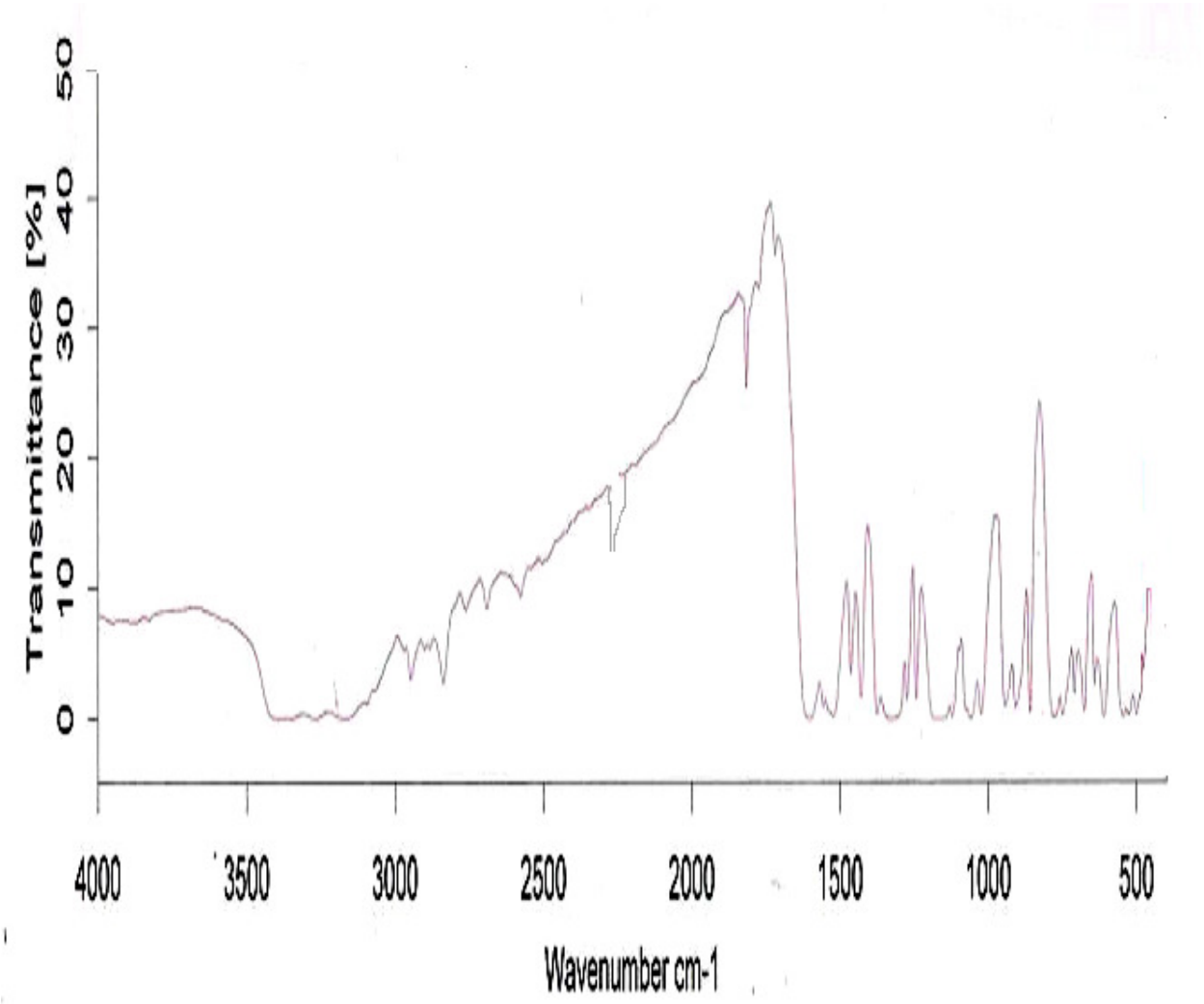
Table 6: Characteristic of IR absorption of HYDROCHLOROTHIAZIDE.

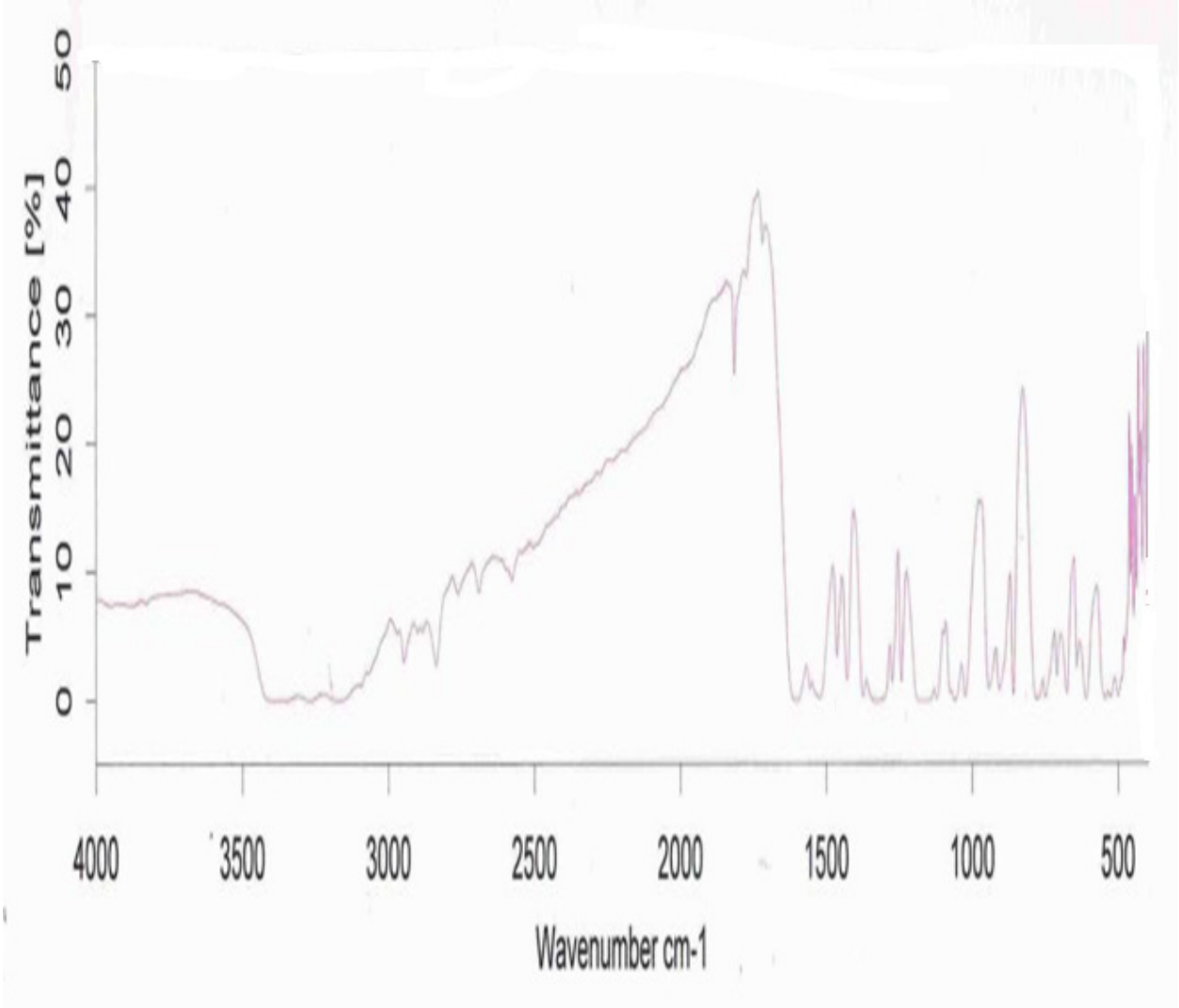
Frequency (cm^{-1})	Groups Assigned
3370-3270	NH or NH2
3030	=CH Aromatic
1600-1550-1520	Hetrocyclic Ring
1600-1450	C=C Aromatic
1370-1335	Asymmetric(SO2)
1180-1160	Symmetric(SO2)
789	(CL_2)

Table 7: Characteristics of IR absorption of Formulation batch B_8.

Frequency (cm^{-1})	Groups Assigned
1600-1450	C=C Aromatic
1370-1335	Asymmetric(SO2)
1180-1160	Symmetric(SO2)
789	(CL_2)

There is no changes in FTIR peaks indicates that no interaction between drug and excipients.


Transmittance [%]
0
10
20
30
40
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1



6.4. EVALUATION OF BUCCAL FILMS:

The prepared formulation were subjected to various physico chemical evaluation test, such as percentage moisture absorption ,percentage moisture loss, swelling index and time taken for maximum swelling, water vapour transmission rate, folding endurance, drug content uniformity, thickness and bioadhesive strength. The films were also subjected to *invitro* dissolution studies.

The physico chemical evaluation of the formulation batch B8 have shown different physical characteristics of the formulation changed according to the nature and composition of polymer.

Physical characteristics:

The Physical characteristics of the buccal films are given in Table:8

Thickness **:** The thicknesses of the tablets were found to be 0.426-0.785 mm.

Drug Content **:** Drug content in the buccal films were within the limits.

Surface pH **:** As an acidic or alkaline surface pH may cause irritation to the buccal mucosa, the buccal films are formulated to have surface pH as close to neutral as possible within salivary pH. The surface pH of all the formulations was close to neutral pH and hence, these formulations may not cause any irritation in the buccal cavity.

The formulation batch B8 (HPMC,HEC,CARBOPOL), have shown highest % moisture absorption due to high hydrophilicity of the hydrophilic polymer.

The formulation B1 to B3 Shows less percentage moisture absorption , which may be due to the HPMC polymer.

Table 8: PHYSICO CHEMICAL EVALUATION OF BUCCAL FILMS OF HYDROCHLOROTHIAZIDE

BATCH CODE	P.M.A (±SD) (%)	P.M.L (±SD) (%)	SWELING INDEX (±SD) (%)	WATER VAPOUR TRANSMISSION RATE (±SD) (mg/cm²/h)	THICKNESS (±SD) (mm)	FOLDING ENDURANCE (±SD)	DRUG CONTENT (±SD) (mg)
B1	10.87 ±0.031	15.12± 0.53	112.61± 0.57	0.887× 10^{-3}±0.006	0.426±0.001	64±1.12	25.53±0.2
B2	25.04± 0.35	21.03± 0.54	127.79± 0.028	1.475×10^{-3}±0.002	0.572±0.006	61±1.12	24.78±0.2
B3	18.32 ±0.33	23.42± 1.25	135.85± 0.43	1.916× 10^{-3}±0.001	0.706±0.006	58.126±	25.04±0.5
B4	11.34± 0.05	11.41± 0.24	139.41± 0.38	1.527× 10^{-3}±0.007	0.703±0.007	71±1.56	24.85±0.7
B5	13.79± 0.14	12.27± 0.16	153.24± 0.66	1.67× 10^{-3}±0.003	0.572±0.005	64±1.45	24.94±0.889
B6	24.26± 0.19	22.37± 0.34	148.81± 1.2	1.061× 10^{-3}±0.002	0.675±0.003	60±1.49	24.72±0.001
B7	20.03± 0.19	16.17± 0.28	152.01± 1.36	2.412× 10^{-3}±0.004	0.785±0.009	82±1.0	24.65±0.02
B8	**16.79± 0.14**	**13.27± 0.17**	**156.24± 0.66**	**2.061× 10^{-3}±0.004**	**0.671±0.008**	**67±1.11**	**25.03±0.009**
B9	19.69± 0.41	25.12± 1.21	140.62± 1.23	2.031× 10^{-3}±0.017	0.719±0.006	58±1.19	25.12±0.3

*PMA =Percent Moisture Absorption *PML = Percentage Moisture Loss

6.5. *INVITRO* MUCOADHESIVE STRENGTH MEASUREMENT:

The results of *invitro* mucoadhesive strength measurement of Hydrochlorothiazide buccal films are given in table 9 and figure 10

Table 9: Mucoadhesive strength of different formulation batches.

Formulation Batches	Mucoadhesive strength
B1	21.15
B2	26.7
B3	24.32
B4	25.1
B5	30.15
B6	28.4
B7	25.2
B8	**33.45**
B9	28.42

The mucoadhesive strength of the formulations was found to be a function of the concentration of the polymer.

Among the formulations, batch B8 exhibited maximum mucoadhesive strength followed by those containing HPMC & HEC and which had higher mucoadhesive strength than HPMC.

The order of mucoadhesive strength was,

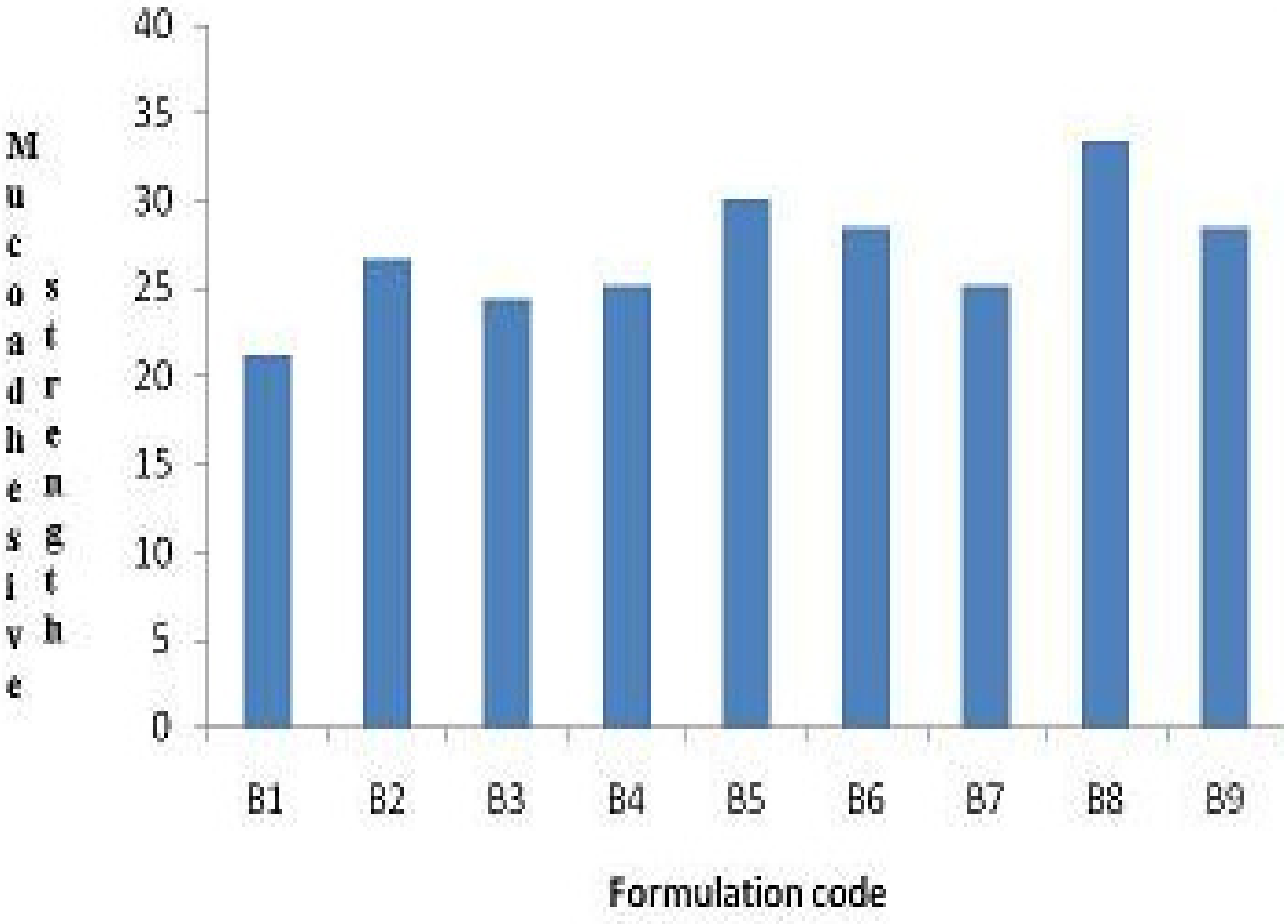
$$B_8 < B_5 < B_9 < B_6 < B_2 < B_7 < B_4 < B_3 < B_1$$

Wetting, interpenetration, and mechanical interlocking between mucous and polymer are the successive stages of mucoadhesion. The strength of mucoadhesion is affected by various factors such as molecular weight of polymers, contact time with mucous, swelling rate of the polymer, and the biological membrane used in the study. This higher mucoadhesion of carbopol 934P may be due to the ionization of carbopol 934P at salivary pH which leads to improved attachment of the device to mucosal surface. The superior quality of Carbopol 934P as

bioadhesive polymer as compared to HPMC K4M and K15M has also been revealed in other studies.

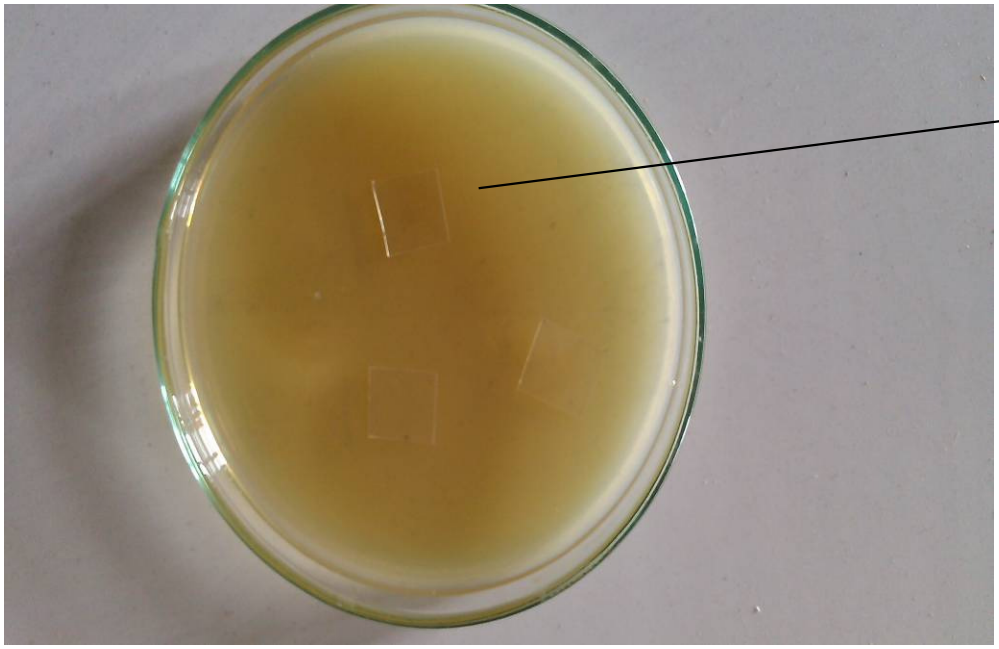
FIG7: Photograph of modified apparatus for *invitro* mucoadhesive strength.

FIG8: Mucoadhesive strength of different formulation batches.



MICROBIAL COUNT

FIG9: Nutrient Agar Medium (control)

FIG10:T 

est

The nutrient agar in petri plates were examined for microbial growth after 24 h of incubation there was no changes observed .

6.6: *INVITRO* DRUG RELEASE STUDY:

As the salivary pH is in the range of 5.8-7.4 and in many studies of buccal drug delivery systems, pH 6.8 phosphate buffer has been used as drug release medium, so the same has been selected for the present study. *invitro* release profiles of hydrochlorothiazide was done formulation from B1 –B9 mucoadhesive table are presented.

Table 10: *INVITRO* DRUG RELEASE DATA FOR B1(3%HPMC)

TIME	% DRUG RELEASE	CUMULATIVE % DRUG RELEASE
10	20	20
20	38.3	38.5
30	46.4	46.78
40	53.6	54.17
50	65.6	66.14
60	70.4	71.61

Figure 11: ***INVITRO* DRUG RELEASE DATA FOR B1**

(3%HPMC)

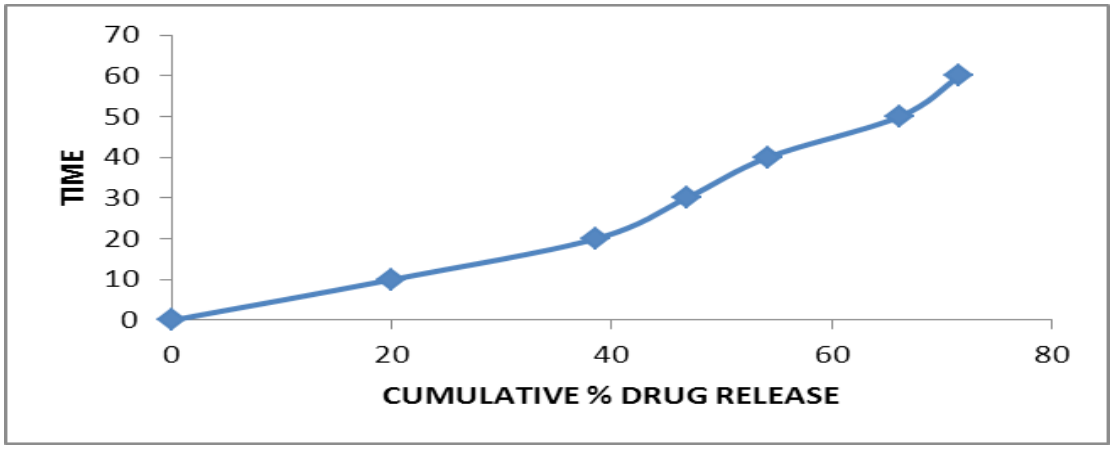


Table 12: *INVITRO* DRUG RELEASE DATA FOR B2 (4%HPMC)

TIME	%DRUG RELEASE	CUMULATIVE % DRUG RELEASE
10	**23**.2	**23.2**

20	**35.2**	**35.43**
30	**47.2**	**47.55**
40	**62.4**	**62.87**
50	**71.2**	**71.83**
60	**84**	**84.72**

FIG 13: *INVITRO* DRUG RELEASE DATA FOR B2 (4%HPMC)

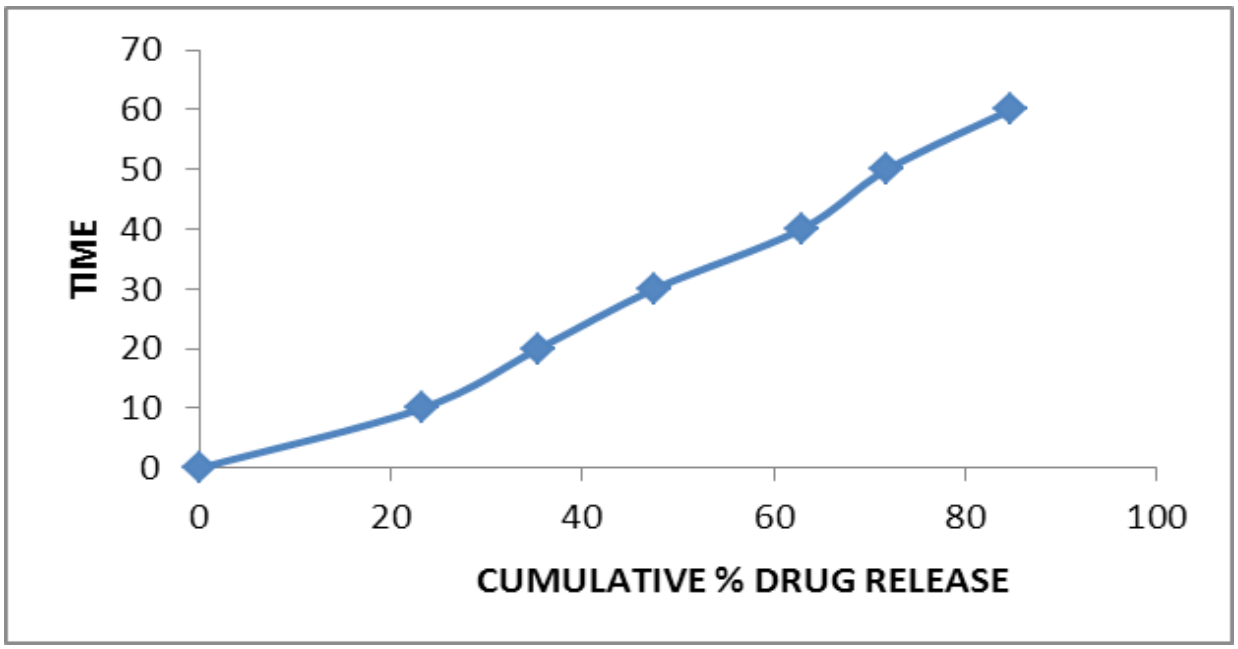


Table 13:*INVITRO* DRUG RELEASE DATA FOR B3

(5%HPMC: 2%HEC)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	11.2	11.2
20	30.4	30.51
30	42.4	42.70
40	57.6	58.13
50	69.6	70.13
60	74.4	75.10
70	81.6	82.35

FIG 13:*INVITRO* DRUG RELEASE DATA FOR B3

(5%HPMC: 2%HEC)

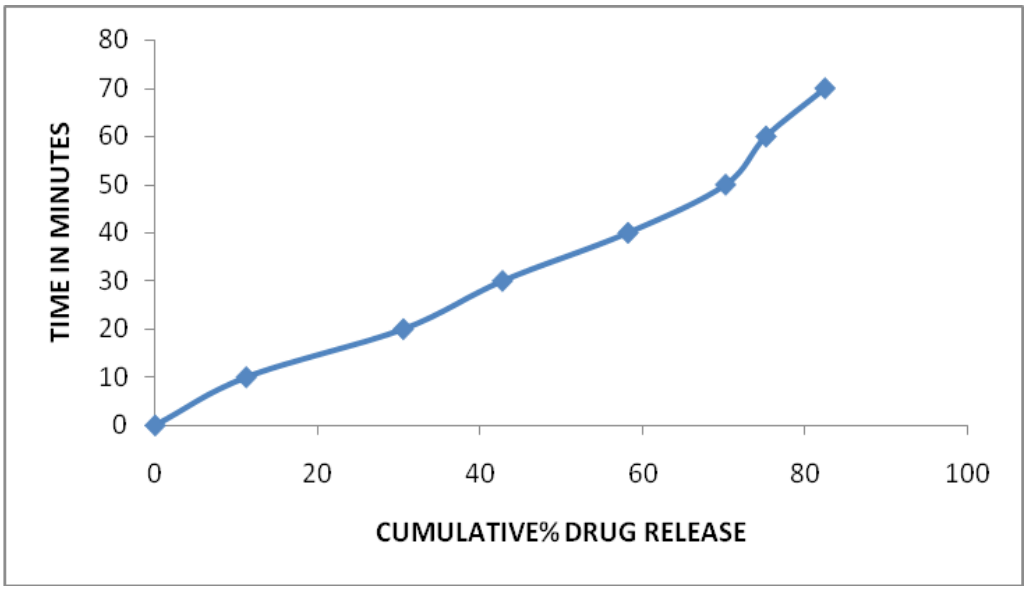


Table 14: *INVITRO* DRUG RELEASE DATA FOR B4

(4%HPMC:2% HEC)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	27.2	27.2
20	39.2	39.29
30	54.4	54.79
40	63.2	63.75
50	71.2	71.84
60	80	80.72
70	89.6	90.41
80	89.6	90.50

FIG 14: *INVITRO* DRUG RELEASE DATA FOR B4

(4%HPMC:2% HEC)

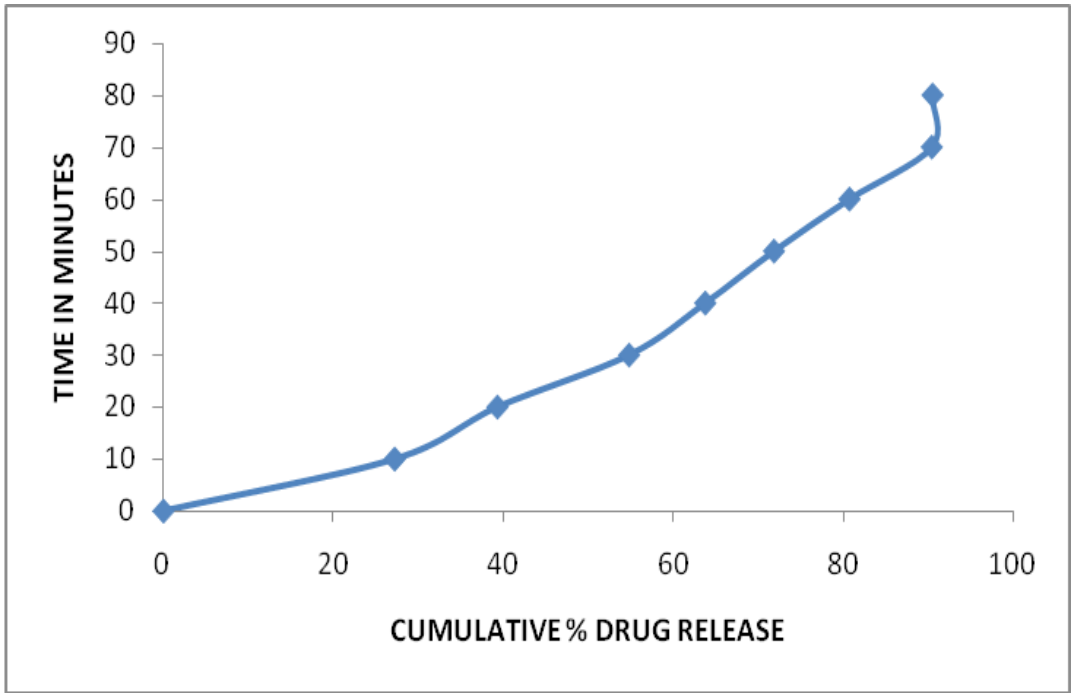


Table 15: ***INVITRO*** **DRUG RELEASE DATA FOR B5**

(4%HPMC:3% HEC)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	21.6	21.6
20	37.60	37.82
30	48	48.38
40	63.2	63.68
50	68	68.68
60	74.4	75.08
70	81.6	82.35
80	90.4	91.92
90	92	92.91

FIG 15: ***INVITRO*** **DRUG RELEASE DATA FOR B5**

(4%HPMC:3% HEC)

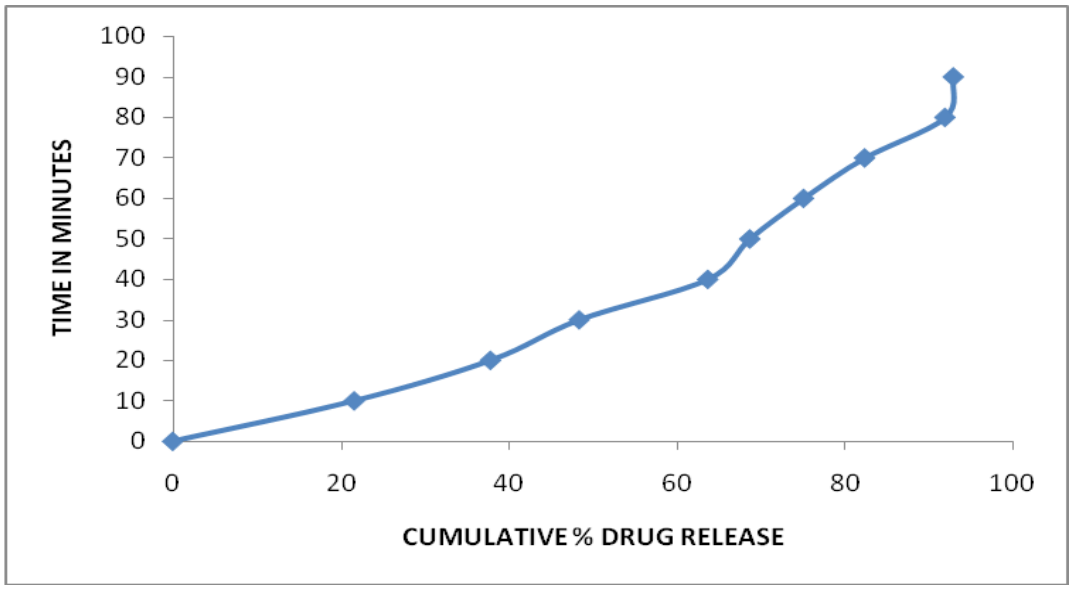


Table 16: *INVITRO* DRUG RELEASE DATA FOR B6

(4%HPMC:4% HEC)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	21.6	21.6
20	34.4	34.62
30	45.6	45.95
40	49.6	50.06
50	56	56.50
60	61.6	62.10
70	69.6	63.12
80	77.6	78.13
90	84	85.78

FIG 16:***INVITRO*** **DRUG RELEASE DATA FOR B6**

(4%HPMC:4% HEC)

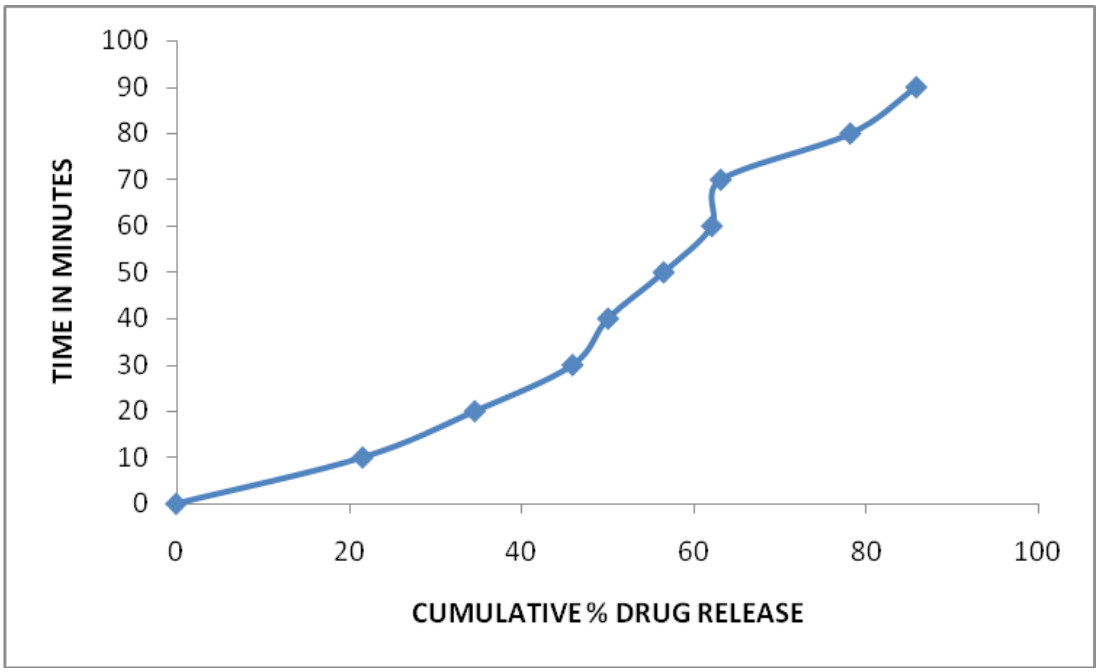


Table 17: *INVITRO* DRUG RELEASE DATA FOR B7

(4%HPMC: 3%HEC:1% CARBOPOL)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	20	20
20	36	36.20
30	41.6	41.96
40	49.6	50.02
50	54	54.50
60	60	60.54
70	63.2	63.80
80	69	69.64
90	72	72.70
100	78.4	79.13
110	81.6	82.39
120	85.6	86.22

FIG 17:***INVITRO*** **DRUG RELEASE DATA FOR B7**

(4%HPMC:3% HEC: 1%CARBOPOL)

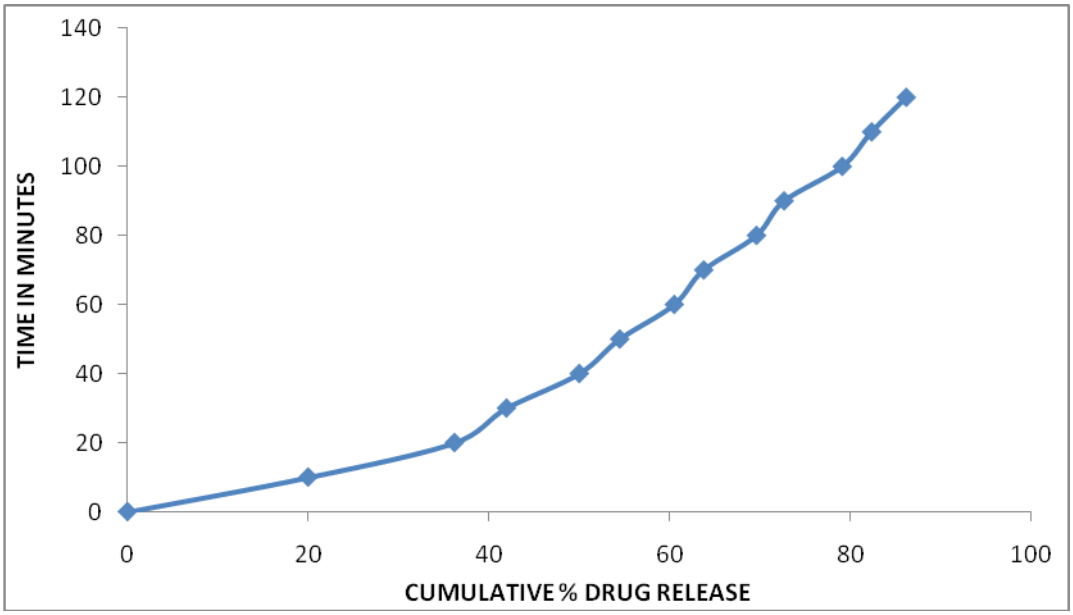


Table 18: *INVITRO* DRUG RELEASE DATA FOR B8

(4%HPMC:3% HEC: 2%CARBOPOL)

TIME	%DRUG RELEASE	CUMULATIVE% DRUG RELEASE
10	23.2	23.2
20	32	32.23
30	39.2	39.52
40	48	48.79
50	53.6	54.08
60	60	60.54
70	64	64.60
80	69.6	70.25
90	73.6	74.30
100	86.4	87.14
110	92	92.87
120	98.4	99.27

FIG 18:*INVITRO* DRUG RELEASE DATA FOR B8

(4%HPMC:3% HEC:2% CARBOPOL)

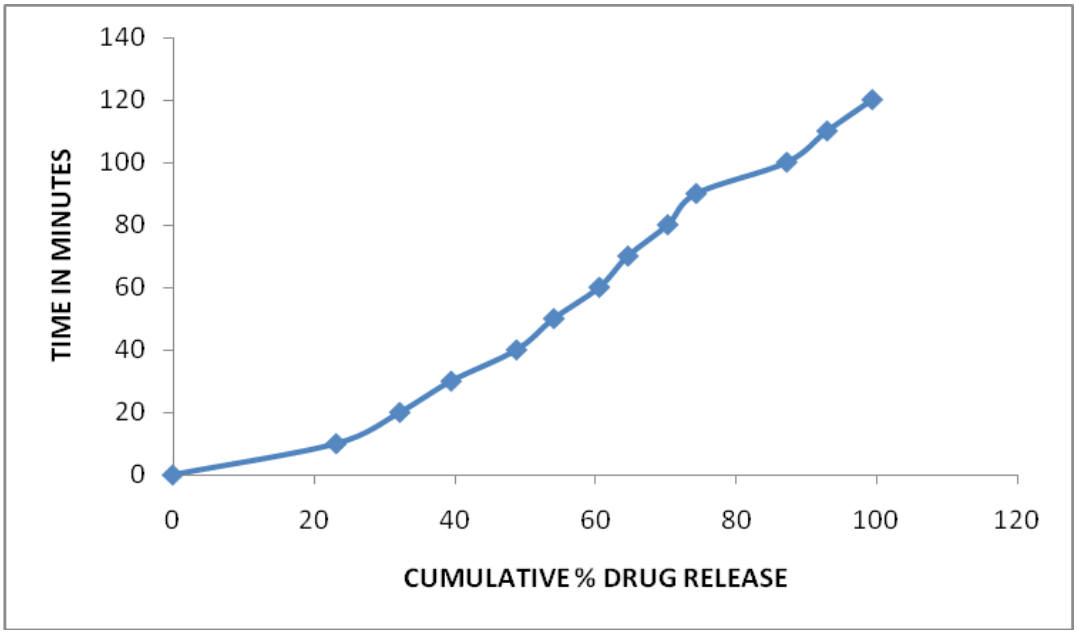


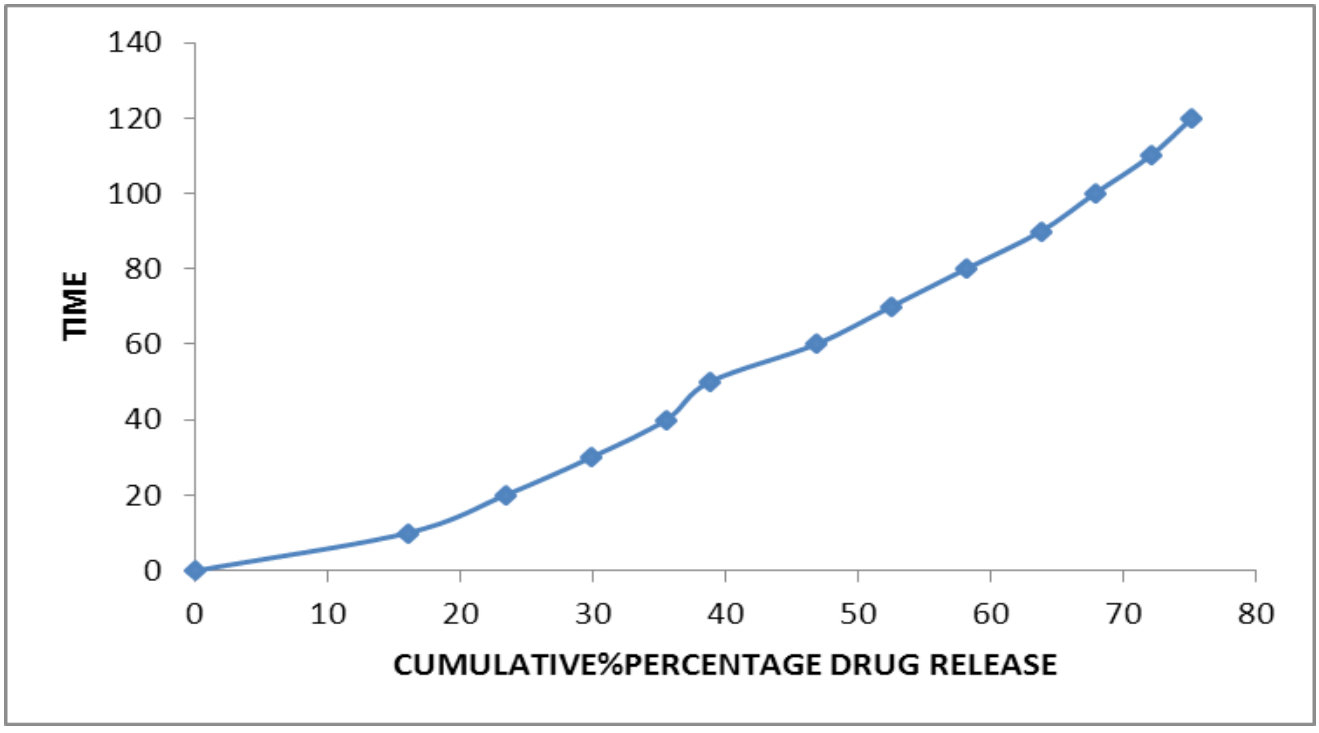
Table 19: *INVITRO* DRUG RELEASE DATA FOR B9

(4%HPMC:3% HEC:3% CARBOPOL: GLYCERIN)

TIME	%DRUG RELEASE	CUMULATIVE%PERCENTAGE DRUG RELEASE
10	16	16
20	23.2	23.36
30	29.6	29.83
40	35.2	35.50
50	38.4	38.75
60	46.4	46.79
70	52	52.47
80	57.6	58.12
90	63.2	63.78
100	67.2	67.84
110	71.2	72.04
120	74.4	75.16

FIG 20:*INVITRO* DRUG RELEASE DATA FOR B9

(4%HPMC:3% HEC:3% CARBOPOL)



***INVITRO* DRUG RELEASE STUDIES:**

The *invitro* release profiles of hydrochlorothiazide from formulation batch B_1-B_9 buccal films.

The release was found to depend on the proportion of Carbopol 934P, HPMC and HEC. The formulations containing(B8) carbopol exhibited higher cumulative amount of drug release. This may be due to the ionization of Carbopol 934P at pH environment of dissolution medium. Ionization of Carbopol leads to the development of negative charges along the backbone of the polymer. Repulsion of like charges uncoils the polymer into an elongated structure, leading to slightly higher uptake of water. This water uptake leads to considerable swelling of polymer. The continued swelling causes the drug to diffuse from formulations at faster rate.

In Formulation B1,B2,B3 (HPMC) the *in vitro* drug release were 71.61% at 1 h, 84% after 1 h and 82.35% after 1 h10 min respectively .The formulation B2 has higher release compared to B1 &B3. So the HPMC 4% is optimum to make the formulation with HEC 2 to 4%.

In formulation B4,B5,B6 the *invitro* drug release were 90.54% after 1h20min , 92.91%% at 1h 30min and 85.78% after 1h30min respectively. The hydrophilic polymer is not efficient, hence the formulation B7 to B9 was formulated with carbopol 1 to 3 %. The *invitro* drug release was 86.22% after 2 h, 99.27% after 2 h ,74.16% after 2 h respectively.

In B8 formulation *invitro* dissolution study of drug along with polymer (HPMC,HEC,Carbopol) show higher rate of % release of drug . Further increase in concentration of polymer in B9 formulation the drug release was decreased, From the above report formulation batch B8 was optimized,and further studies was carried out.

6.7. KINETICS OF DRUG RELEASE

FIG 21: ZERO ORDER PLOT FOR BATCH B8

(4%HPMC:3% HEC: 2%CARBOPOL)

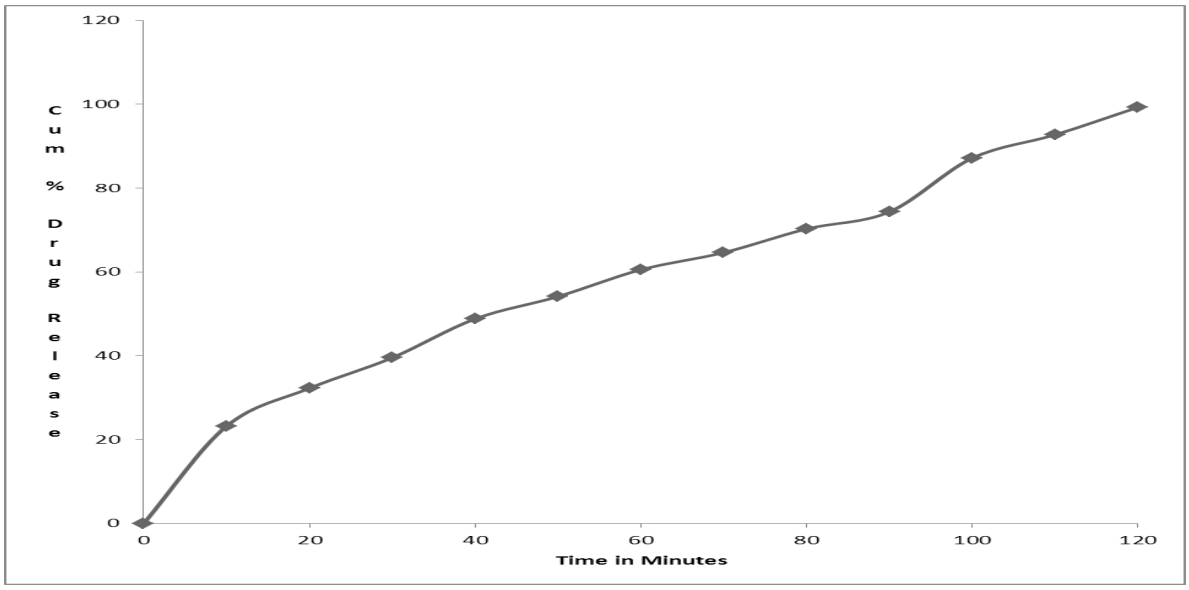


FIG 22:FIRST ORDER PLOT –BATCH B8

4%HPMC:3% HEC: 2%CARBOPOL)

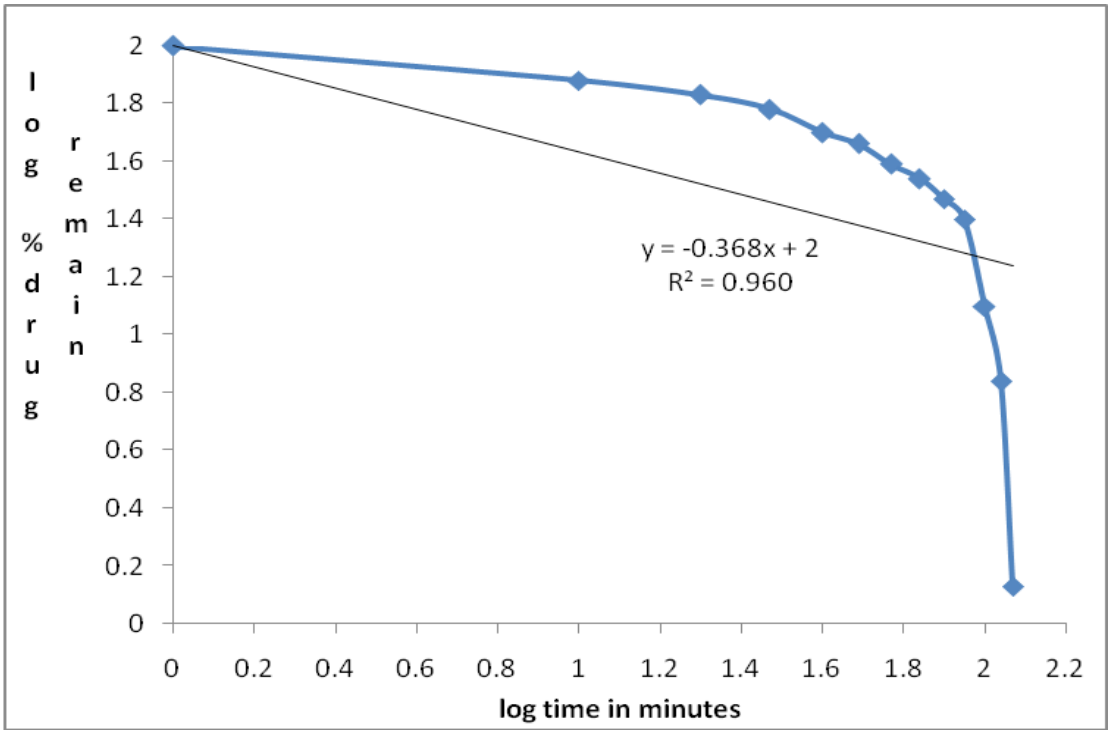


FIG 23:HIGUCHIS PLOT –BATCH B8

(4%HPMC:3% HEC: 2%CARBOPOL)

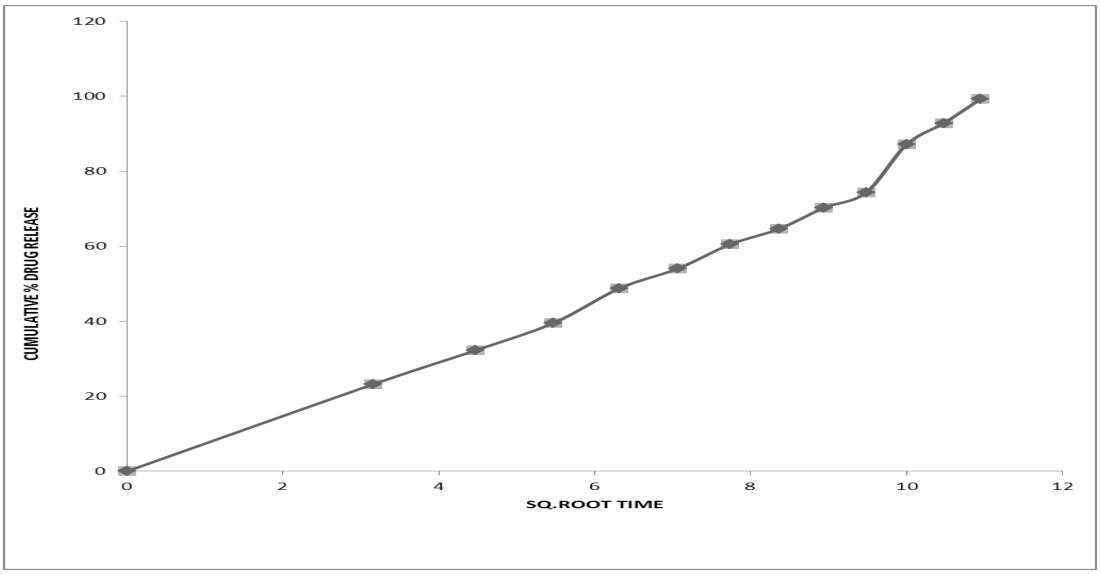
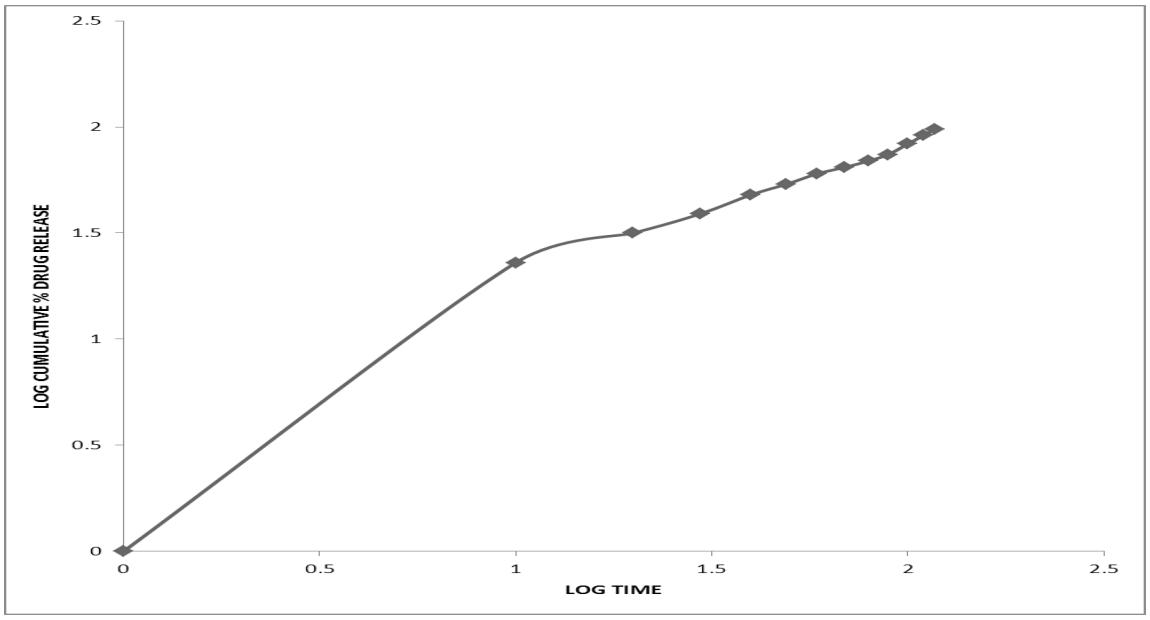


FIG 24:**PEPPAS PLOT –BATCH B8**

(4%HPMC: 3%HEC:2% CARBOPOL)



The fitting of the release data in first-order, Korsmeyer Peppas and Higuchi's model are shown in FIG 21-24.

The release rate was found to follow first-order release shown by the best fitting straight line. This behavior has been confirmed by the linear plots obtained with Korsemeyer Peppas treatment of the data. Further the noncompliance of the data with Fickian diffusion though matrix is indicated by non linear Higuchi plots.

6.8. *EXVIVO* DIFFUSION STUDIES

An *exvivo* diffusion study of hydrochlorothiazide was carried out. Fresh goat cheek pouch membrane was tied to one end of an open cylinder, which acts as a donor compartment . The film should be placed in such a way that it should be stuck on the mucous membrane. The receptor compartment was filled with isotonic phosphate buffer pH 6.8. The assembly was

maintained at 37^0C and stirred magnetically. Samples were withdrawn at 10 min intervals for 2 h and analysed using UV- Spectrophotometer at 274nm.

Table 19*: EXVIVO* DIFFUSION STUDIES FOR B8
(4%HPMC:3% HEC: 2%CARBOPOL)

Time in minutes	Drug release in Mg	Cumulative % drug release
10	2.44	12.4
20	3.66	18.6
30	4.42	22.4
40	6.26	31.8
50	8.40	42.6
60	9.81	49.8
70	10.64	54
80	12.45	63.2
90	13.91	70.6
100	15.05	76.4
110	16.07	81.6
120	16.78	85.2

***EXVIVO* DIFFUSION STUDIES FOR B8**

FIG 25: (4%HPMC: 3%HEC: 2%CARBOPOL)

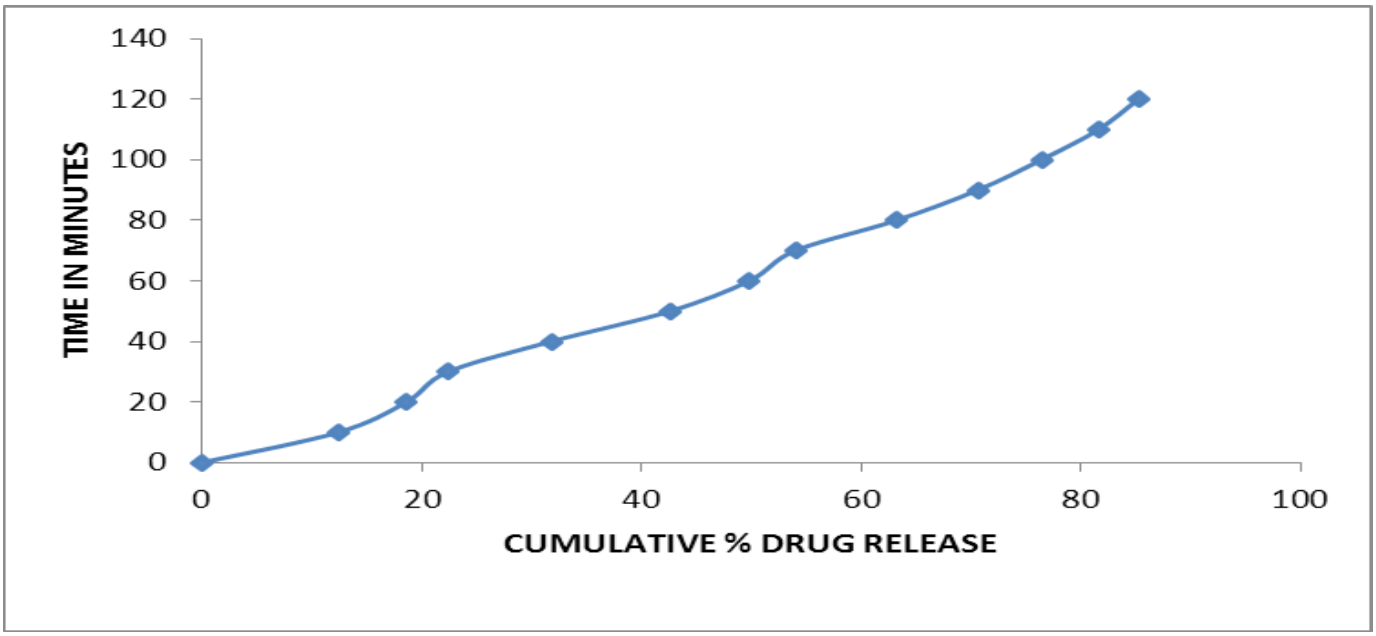


Table: 20

Time In Minutes	Cumulative % drug release		
	Invitro drug release studies	*Exvivo* drug release studies	*Invivo* drug release studies
10	23.2	12.4	4.4
20	32.23	18.6	8.08
30	39.52	22.4	17.44
40	48.79	31.8	26.26
50	54.08	42.6	33.32
60	60.54	49.8	44.47
70	64.60	54	57.65
80	70.25	63.2	66.45
90	74.30	70.6	77.79
100	87.14	76.4	84.46
110	92.87	81.6	89.41
120	99.27	85.2	96.22

***Invitro* permeation study.**

Porcine membrane is identical to human buccal mucosa, so porcine membrane was used for *invitro* permeation studies. Very strong bioadhesion could damage the epithelial lining of the buccal mucosa.

***IN VIVO* DRUG RELEASE STUDIES**

METHODS

A healthy rabbit weighing 2.5 to 3kg was taken which was already checked for absence of any diseases. The fore limbs and hind limbs were tied into the iron of the mini operation table; so that rabbit was in dorsal position. The prepared film having the size of 1cm containing 20 mg of hydrochlorothiazide was placed in buccal membrane with the help of clip. Dextrose solution was transfused continuously though out the period of study. Periodically 1ml blood samples were taken using a syringe which already contained 1ml of 3.8% sodium citrate solution to prevent blood clotting. These blood samples were subjected for centrifuging at 2,200 rpm for about 20minites. 1ml of supernatant liquid was taken from this and after suitable dilution; these samples were analyzed at 274nm using spectrophotometer.

Table21: *INVIVO* DRUG RELEASE STUDIES – BATCH 8

(4%HPMC:3% HEC: 2%CARBOPOL)

Time in minutes	Amount of drug release	Precentage drug release
10	0.4	4.4
20	0.80	8.08
30	1.74	17.44
40	2.62	26.26
50	3.12	33.32
60	4.42	44.47
70	5.7	57.65
80	6.8	66.45
90	7.81	77.79
100	8.23	84.46
110	8.25	89.41
120	9.1	96.22

FIG 27:*IN VIVO* DRUG RELEASE STUDIES – BATCH 8

(4%HPMC:3% HEC:2% CARBOPOL)

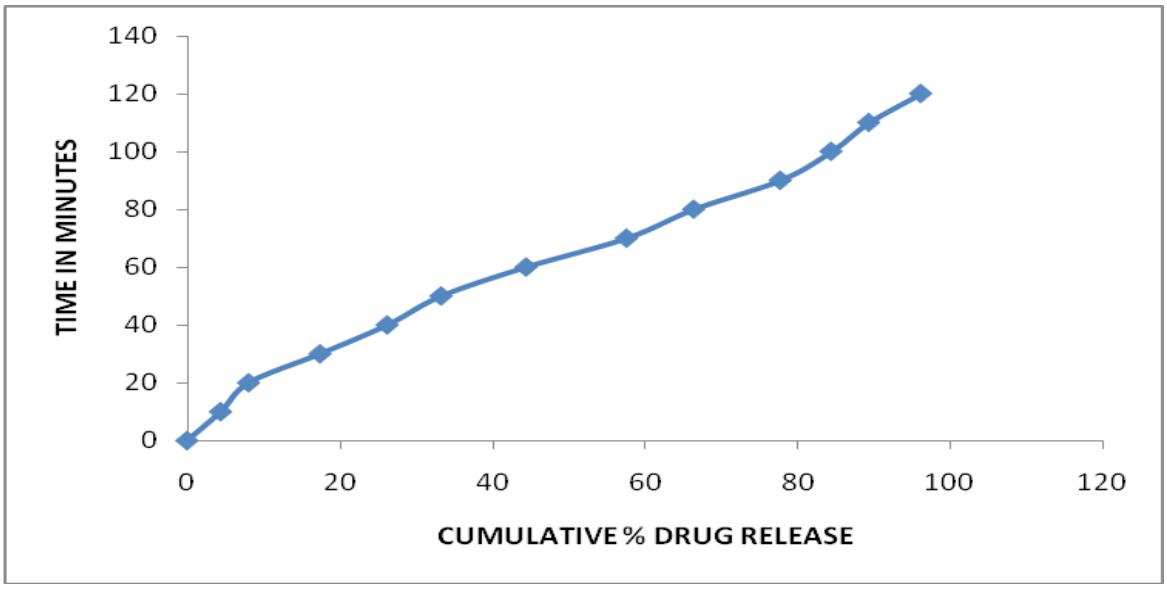


Table 22: COMPARISON OF *INVITRO* - *EXVIVO* DRUG RELEASE STUDIES

Time in minutes	*In vitro* cumulative % drug release	*Exvivo* cumulative % drug release
10	23.2	12.4
20	32.23	18.6
30	39.52	22.4
40	48.79	31.8
50	54.12	42.6
60	60.52	49.8
70	64.6	54
80	70.25	63.2
90	74.32	70.6
100	87.14	76.4
110	92.72	81.6
120	99.27	85.2

FIG28:*IN VIVO– IN VITRO* CORRELATION PLOT

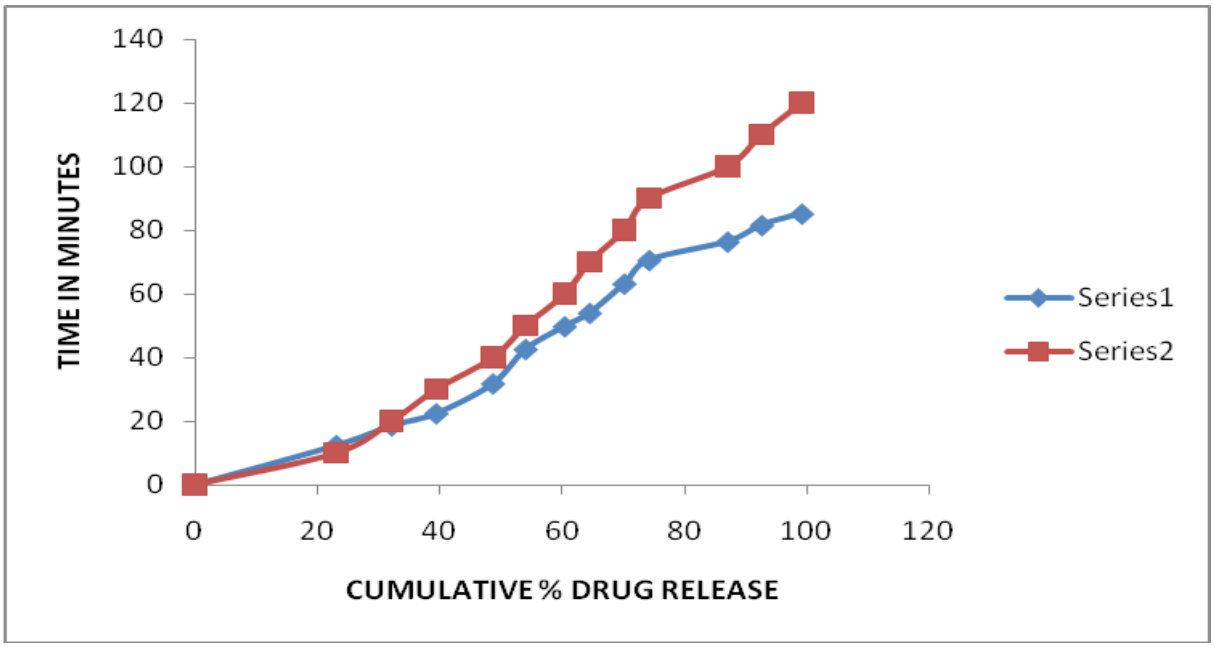
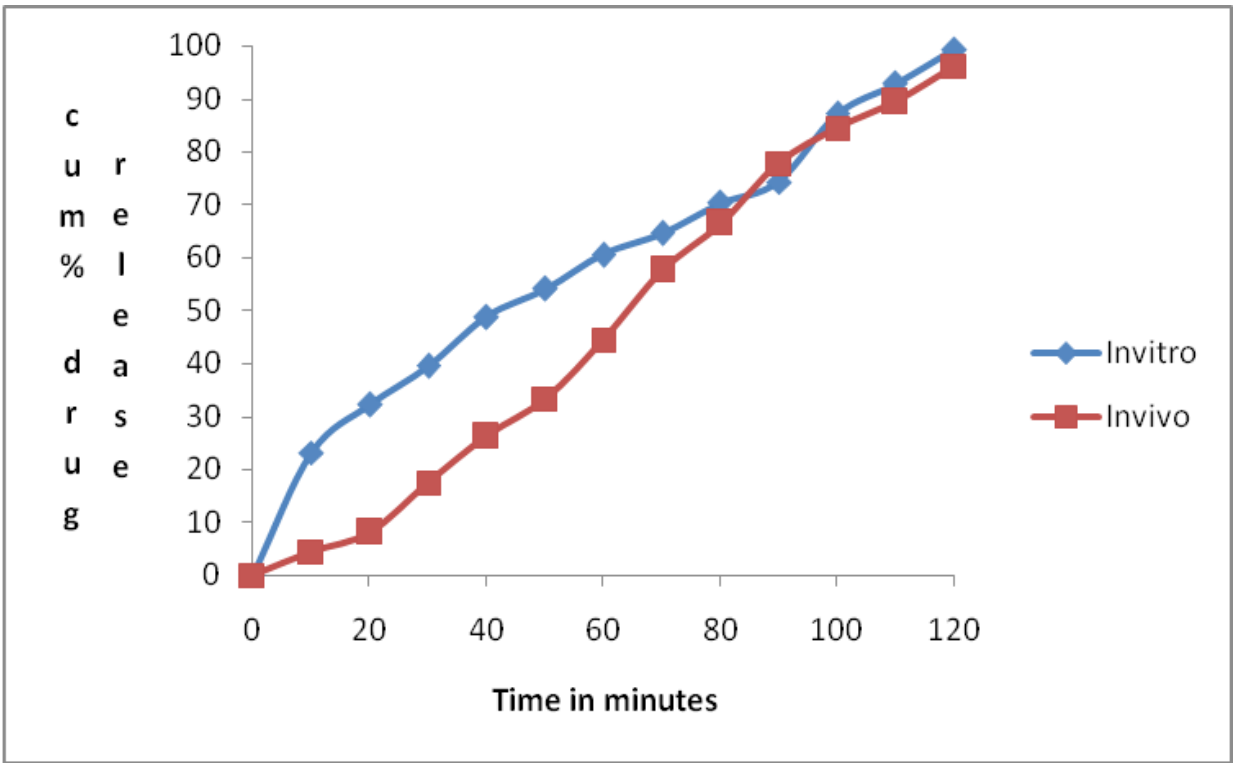


Table 23: COMPARISON OF *INVITRO – INVIVO* DRUG RELEASE STUDIES

Time in Minutes	Cumulative % drug release (*INVITRO*)	Cumulative % drug release *(INVIVO)*
10	23.2	4.4
20	32.23	8.08
30	39.52	17.44
40	48.79	26.26
50	54.08	33.32
60	60.54	44.47
70	64.6	57.65
80	70.25	66.45
90	74.3	77.79
100	87.14	84.46
110	92.87	89.41
120	99.27	96.22

FIG 28:*IN VIVO– IN VITRO* CORRELATION PLOT



7.9. STABILITY STUDIES

PHYSICAL PARAMETERS:

The optimized formulation batch B8 did not show any significant changes in the physical parameters and results were within limits. The results were shown in table 24.

Sl. No	Parameters	4^0C			Room Temperature			40^0C/ 75% RH (Stability chamber)		
		Initial	7 Days	15 Days	30 Days	7 Days	15 Days	30 Days	7 Days	15 Days	30 Days
1	*INVITRO* % drug release	0	98.7	97	97	98.5	95	92	95	89	85
2	Drug Content (%)	99.1	99.1	98.6	98.8	99.1	98.1	98.1	98.8	98.3	98.1
3	Surface pH	6.8	6.8	6.8	6.8	6.8	6.8	6.7	6.8	6.7	6.7

Table 24

The optimized formulation batch B8 did not show any significant changes in drug release profile after a period of 3 months. Hence it can be concluded that the optimized batch B8 is stable at an accelerated storage conditions.

7. CONCLUSION

Efforts should be made to develop standard *invitro* method to characterize and compare different material and formulation in terms of their capability to promote drug absorption via the buccal route.

Buccal adhesive systems offer innumerable advantages in terms of accessibility, administration and withdrawal, retentively, low enzymatic activity, economy and high patient compliance.

Adhesions of these drug delivery devices to mucosal membranes lead to an increased drug concentration gradient at the absorption site and therefore improve bioavailability of systemically delivered drugs.

The results of the study show that therapeutic levels of Hydrochlorothiazide can be delivered through buccal, formulation batch B8 show good swelling, a convenient residence time and promising controlled drug release thus seems to be a potential candidate for the development of buccal film for effective therapeutic use. *Invivo* studies need to be designed and executed to substantiate further *invitro - invivo* correlation.

The main advantages of the buccal route of administration over the traditional per oral route are that drug degradation in the stomach, first-pass metabolism is avoided, and therapeutic drug levels of drug can be achieved rapidly. The future direction of buccal adhesive drug delivery lies in vaccine formulations and delivery of small proteins/ peptides.

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